Guidance for Industry

Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice

DRAFT GUIDANCE

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Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
Office of Regulatory Affairs (ORA)

August 2003
Pharmaceutical CGMPs

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TABLE OF CONTENTS

I. INTRODUCTION 1

II. BACKGROUND 2

A. Regulatory Framework 2

B. Technical Framework 2

III. SCOPE 3

IV. BUILDINGS AND FACILITIES 4

A. Critical Area – Class 100 (ISO 5) 5

B. Supporting Clean Areas 6

C. Clean Area Separation 7

D. Air Filtration 7

1. Membrane *7*
2. High-Efficiency Particulate Air (HEPA) *8*

E. Design 10

V. PERSONNEL TRAINING, QUALIFICATION, & MONITORING 13

A. Personnel 13

B. Laboratory Personnel 15

C. Monitoring Program 15

VI. COMPONENTS AND CONTAINER/CLOSURES 16

A. Components 16

B. Containers/Closures 18

1. Preparation *18*
2. Inspection of Container Closure System *19*

VII. ENDOTOXIN CONTROL 20

VIII. TIME LIMITATIONS 21

IX. VALIDATION OF ASEPTIC PROCESSING AND STERILIZATION 21

A. Process Simulations 21

1. Study Design *22*
2. Frequency and Number of Runs *23*
3. Duration of Runs *23*
4. Size of Runs *24*
5. Line Speed *24*
6. Environmental Conditions *24*
7. Media *25*
8. Incubation and Examination of Media-Filled Units *25*
9. Interpretation of Test Results *26*

B. Filtration Efficacy 27

C. Sterilization of Equipment and Container and Closures **29**

1. Sterilizer Qualification and Validation *29*
2. Equipment Controls and Instrument Calibration *30*

X. LABORATORY CONTROLS **32**

A. Environmental Monitoring **33**

1. General Written Program *33*
2. Establishing Levels and a Trending Program *34*
3. Sanitization Efficacy *34*
4. Monitoring Methods *35*

B. Microbiological Media and Identification **36**

C. Prefiltration Bioburden **36**

D. Alternate Microbiological Test Methods **37**

E. Particle Monitoring **37**

XI. STERILITY TESTING **38**

A. Choice of Methods **39**

B. Media **39**

C. Personnel **39**

D. Sampling and Incubation **40**

E. Investigation of Sterility Positives **40**

XII. BATCH RECORD REVIEW: PROCESS CONTROL DOCUMENTATION **43**

APPENDIX 1: ASEPTIC PROCESSING ISOLATORS **45**

APPENDIX 2: BLOW-FILL- SEAL TECHNOLOGY **50**

APPENDIX 3: PROCESSING PRIOR TO FILLING AND SEALING OPERATIONS **53**

REFERENCES **55**

RELEVANT GUIDANCE DOCUMENTS **56**

GLOSSARY **57**

Contains Nonbinding Recommendations

Guidance for Industry[1]
Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This draft guidance is intended to help manufacturers meet the requirements in the Agency's current good manufacturing practice (CGMP) regulations (2l CFR parts 210 and 211) when manufacturing sterile drug and biological products using aseptic processing. This guidance, when finalized, will replace the 1987 *Industry Guideline on Sterile Drug Products Produced by Aseptic Processing*. This revision updates and clarifies the 1987 guidance.

For sterile drug products subject to a new or abbreviated drug application (NDA or ANDA), this guidance document should be read in conjunction with the 1994 guidance on the content of sterile drug applications, entitled *Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products*. The 1994 submission guidance describes the types of information and data that should be included in drug applications to demonstrate the efficacy of a manufacturer's sterilization process. This draft guidance compliments the 1994 guidance by describing procedures and practices that will help enable a sterile drug manufacturing facility to meet CGMP requirements relating, for example, to facility design, equipment suitability, process validation, and quality control.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

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[1] This guidance was developed by the Office of Compliance in the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER) and the Office of Regulatory Affairs (ORA).

The text boxes included in this guidance discuss specific sections of parts 210 and 211 of the Code of Federal Regulations (CFR), which address current good manufacturing practice for drugs. The intent of including the citations in the text boxes is to aid the reader by providing a portion of an applicable regulation being addressed in the guidance. The citations included in the text boxes are not intended to be exhaustive. Readers of this document should reference the complete CFR to ensure that they have complied, in full, with all relevant sections of the regulations.

II. BACKGROUND

This sections describes briefly both the regulatory and technical reasons why the Agency is developing this guidance document.

A. Regulatory Framework

This draft guidance pertains to current good manufacturing practice (CGMP) regulations (21 CFR parts 210 and 211) when manufacturing sterile drug and biological products using aseptic processing. For biological products regulated under 21 CFR parts 600 through 680, sections 210.2(a) and 211.1(b) provide that where it is impossible to comply with the applicable regulations in both parts 600 through 680 and parts 210 and 211, the regulation specifically applicable to the drug product in question shall apply. In the event that it is impossible to comply with all applicable regulations in these parts, the regulations specifically applicable to the drug in question shall supersede the more general.

B. Technical Framework

There are basic differences between the production of sterile drug products using aseptic processing and production using terminal sterilization.

Terminal sterilization usually involves filling and sealing product containers under high-quality environmental conditions. Products are filled and sealed in this type of environment to minimize the microbial content of the in-process product and to help ensure that the subsequent sterilization process is successful. In most cases, the product, container, and closure have low bioburden, but they are not sterile. The product in its final container is then subjected to a sterilization process such as heat or irradiation.

In an aseptic process, the drug product, container, and closure are first subjected to sterilization methods separately, as appropriate, and then brought together.[2] Because there is no process to sterilize the product in its final container, it is critical that containers be filled and sealed in an extremely high-quality environment. Aseptic processing involves more variables than terminal sterilization. Before aseptic assembly into a final product, the individual parts of the final product are generally subjected to several sterilization processes. For example, glass containers are subjected to dry heat sterilization; rubber closures are subjected to moist heat sterilization; and liquid dosage forms are subjected to sterile filtration. Each of these aseptic manufacturing processes requires thorough validation and control. Each process also could introduce an error that ultimately could lead to the distribution of a contaminated product. Any manual or mechanical manipulation of the sterilized drug, components, containers, or closures prior to or during aseptic assembly poses the risk of contamination and thus necessitates careful control. A terminally sterilized drug product, on the other hand, undergoes a single sterilization process in a sealed container, thus limiting the possibilities for error.[3]

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[2] Due to their nature, certain products are aseptically processed at an earlier stage in the process, or in their entirety. Cell-based therapy products are an example. All components and excipients for these products are rendered sterile, and release of the final product is contingent on determination of sterility. See Appendix III.

Manufacturers should have a keen awareness of the public health implications of distributing a nonsterile product. Poor CGMP conditions at a manufacturing facility can ultimately pose a life-threatening health risk to a patient.

III. SCOPE

This guidance document discusses selected issues and does not address all aspects of aseptic processing. For example, the guidance addresses primarily finished drug product CGMP issues while only limited information is provided regarding upstream bulk processing steps. This guidance updates the 1987 guidance primarily with respect to personnel qualification, cleanroom design, process design, quality control, environmental monitoring, and review of production records. The use of isolators for aseptic processing is also discussed.

Although this guidance document discusses CGMP issues relating to the sterilization of components, containers, and closures, terminal sterilization of drug products is not addressed. It is a well-accepted principle that sterile drugs should be manufactured using aseptic processing only when terminal sterilization is infeasible. However, some final packaging may afford some unique and substantial advantage (e.g., some dual-chamber syringes) that would not be possible if terminal sterilization were employed. In such cases, a manufacturer can explore the option of adding adjunct processing steps to increase the level of sterility confidence.

A list of references that may be of value to the reader is included at the conclusion of this document.

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[3] Nearly all drugs recalled due to nonsterility or lack of sterility assurance in the period spanning 1980-2000 were produced via aseptic processing.

IV. BUILDINGS AND FACILITIES

21 CFR 211.42(c) states, in part, that "Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: *** (iii) An air supply filtered through high-efficiency particulate air filters under positive pressure ***; (iv) A system for monitoring environmental conditions; *** (vi) A system for maintaining any equipment used to control the aseptic conditions."

21 CFR 211.46(b) states that "Equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing, or holding of a drug product."

21 CFR 211.46(c) states, in part, that "Air filtration systems, including prefilters and particulate matter air filters, shall be used when appropriate on air supplies to production areas."

As provided for in the regulations, aseptic processing facilities must have separate areas of operation that are appropriately controlled to attain different degrees of air quality depending on the nature of the operation. Design of a given area should be based on satisfying microbiological and particle standards defined by the equipment, components, and products exposed, as well as the particular operation conducted in the area.

Critical areas and support areas of the aseptic processing operation should be classified and supported by microbiological and particle data obtained during qualification studies. Although initial cleanroom qualification should include some assessment of air quality under as-built and static conditions, the final room or area classification should be derived from data generated under dynamic conditions (i.e., with personnel present, equipment in place, and operations ongoing). The aseptic processing facility monitoring program should also assess conformance with specified clean area classifications under dynamic conditions on a routine basis.

The following table summarizes clean area air classifications (Ref. 1).

TABLE 1 - Air Classifications[a]

Clean Area Classification (0.5 um particles/ft^3)	ISO Designation[b]	≥ 0.5 um particles/m^3	Microbiological Active Air Action Levels[c] (cfu/m^3)	Microbiological Settling Plates Action Levels[c,d] (diam. 90mm; cfu/4 hours)
100	5	3,520	1[e]	1[e]
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

a- All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.

b- ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle concentration is equal to Class 100 and approximately equals EU Grade A.

c- Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological levels due to the nature of the operation.

d- The additional use of settling plates is optional.

e- Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

Two clean areas are of particular importance to sterile drug product quality: the critical area and the supporting clean areas associated with it.

A. Critical Area – Class 100 (ISO 5)

A critical area is one in which the sterilized drug product, containers, and closures are exposed to environmental conditions designed to preserve sterility. Activities conducted in this area include manipulations (e.g., aseptic connections, sterile ingredient additions) of sterile materials prior to and during filling and closing operations.

This area is critical because the product is not processed further in its immediate container and is vulnerable to contamination. To maintain product sterility, the environment in which aseptic operations (e.g., equipment setup, filling) are conducted should be of appropriate quality. One aspect of environmental quality is the particle content of the air. Particles are significant because they can enter a product and contaminate it physically or, by acting as a vehicle for microorganisms, biologically (Ref. 2). Particle content in critical areas should be minimized by appropriately designed air handling systems.

Air in the immediate proximity of exposed sterilized containers/closures and filling/closing operations would be of appropriate particle quality when it has a per-cubic-meter particle count of no more than 3520 in a size range of 0.5 micron and larger when counted at representative locations normally not more than 1 foot away from the work site, within the airflow, and during filling/closing operations. This level of air cleanliness is also known as Class 100 (ISO 5). Deviations from this critical area monitoring parameter should be documented as to cause and significance.

Measurements to confirm air cleanliness in aseptic processing zones should be taken with the particle counting probe oriented in the direction of oncoming airflow and at the sites where there is most potential risk to the exposed sterilized product and container-closures. Regular monitoring should be performed during each shift. Nonviable particle monitoring with a remote counting system is generally less invasive than the use of portable particle counting units and provides the most comprehensive data. See Section X.D. Particle Monitoring.

Some powder filling operations can generate high levels of powder particles that, by their nature, do not pose a risk of product contamination. It may not, in these cases, be feasible to measure air quality within the one-foot distance and still differentiate background levels of powder particles from air contaminants. In these instances, air should be sampled in a manner that, to the extent possible, characterizes the true level of extrinsic particle contamination to which the product is exposed. Initial certification of the area under dynamic conditions without the actual powder filling function should provide some baseline information on the nonproduct particle generation of the operation.

Air in critical areas should be supplied at the point of use as HEPA-filtered laminar flow air at a velocity sufficient to sweep particles away from the filling/closing area and maintain unidirectional airflow during operations. The velocity parameters established for each processing line should be justified and appropriate to maintain unidirectional airflow and air quality under dynamic conditions within a defined space (Ref. 3).[4]

Proper design and control should prevent turbulence or stagnant air in the aseptic processing line or clean area. Once relevant parameters are established, airflow patterns should be evaluated for turbulence or eddy currents that can act as a channel or reservoir for the accumulation of air contaminants (e.g., from an adjoining lower classified area). Air pattern analysis or smoke studies should be conducted that demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions. The studies should be well documented with written conclusions, including an evaluation of the impact of aseptic manipulations. Videotape or other recording mechanisms have been found to be useful in assessing airflow initially as well as facilitating evaluation of subsequent equipment configuration changes. However, even successfully qualified systems can be compromised by poor operational, maintenance or personnel practices.

Air monitoring of critical areas should normally yield no microbiological contaminants. Contamination in this environment should receive investigative attention.

B. Supporting Clean Areas

Supporting clean areas can have various classifications and functions. Many support areas function as zones in which nonsterile components, formulated products, in-process materials, equipment, and container/closures are prepared, held, or transferred. These environments should be designed to minimize the level of particle contaminants in the final product and control the microbiological content (bioburden) of articles and components that are subsequently sterilized.

The nature of the activities conducted in a supporting clean area should determine its classification. An area classified at Class 100,000 (ISO 8) would be used for less critical activities (such as initial equipment preparation). The area immediately adjacent to the aseptic processing line should, at a minimum, meet Class l0,000 (ISO 7) standards (see Table 1) under dynamic conditions. Depending on the operation, manufacturers can also classify this area as Class 1,000 (ISO 6) or maintain the entire aseptic filling room at Class 100 (ISO 5).

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[4] A velocity from 0.45 to 0.51 meters/second (90 to 100 feet per minute) is generally established, with a range of plus or minus 20 percent around the setpoint. Higher velocities may be appropriate in operations generating high levels of particulates.

C. Clean Area Separation

Adequately separating areas of operation is an important part of contamination prevention. To maintain air quality in areas of higher cleanliness, it is important to achieve a proper airflow and a positive pressure differential relative to adjacent less clean areas. Rooms of higher air cleanliness should have a substantial positive pressure differential relative to adjacent rooms of lower air cleanliness. For example, a positive pressure differential of at least 12.5 Pascals (Pa)[5] should be maintained at the interface between classified and unclassified areas. This same overpressure should be maintained between the aseptic processing room and adjacent rooms (with doors closed). When doors are open, outward airflow should be sufficient to minimize ingress of contamination, and the time that a door can remain ajar should be strictly controlled (Ref. 4). Pressure differentials between cleanrooms should be monitored continuously throughout each shift and frequently recorded, and deviations from established limits should be investigated.

An adequate air change rate should be established for a cleanroom. For Class 100,000 (ISO 8) supporting rooms, airflow sufficient to achieve at least 20 air changes per hour would be typically acceptable. For areas of higher air cleanliness, significantly higher air change rates will provide an increased level of air purification.

Facility monitoring systems should be established to rapidly detect atypical changes that can compromise the facility's environment. Operating conditions should be restored to established, qualified levels before reaching action levels. For example, pressure differential specifications should enable prompt detection (i.e., alarms) of an emerging low pressure problem to preclude ingress of unclassified air into a classified room.

D. Air Filtration

1. Membrane

A compressed gas should be of appropriate purity (e.g., free from oil and water vapor) and its microbiological and particle quality should be equal to or better than air in the environment into which the gas is introduced. Compressed gases such as air, nitrogen, and carbon dioxide are often used in cleanrooms and are frequently employed in operations involving purging or overlaying.

Membrane filters allow the filtering of compressed gases to meet an appropriate high-quality standard. Membrane filters can be used to produce a sterile compressed gas to conduct operations involving sterile materials, such as components and equipment. For example, sterile membrane filters should be used for autoclave air lines, lyophilizer vacuum breaks, and tanks containing sterilized materials. Sterilized holding tanks and any contained liquids should be held under continuous overpressure to prevent microbial contamination. Safeguards should be in place to prevent a pressure change that can result in contamination due to back flow of nonsterile air or liquid.

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[5] Equal to 0.05 inches of water gauge.

Gas filters (including vent filters) should be dry. Condensate in a gas filter can cause blockage or microbial contamination. Use of hydrophobic filters, as well as application of heat to these filters where appropriate, prevents problematic moisture residues. Filters also should be integrity tested upon installation and periodically thereafter (e.g., including at end of use). Integrity test failures should be investigated, and filters should be replaced at appropriate intervals.

2. High-Efficiency Particulate Air (HEPA)[6]

An essential element in ensuring aseptic conditions is the maintenance of HEPA filter integrity. Leak testing should be performed at installation to detect integrity breaches around the sealing gaskets, through the frames, or through various points on the filter media. Thereafter, leak tests should be performed at suitable time intervals for HEPA filters in the aseptic processing facility. For example, such testing should be performed twice a year for the aseptic processing room. Additional testing may be appropriate when air quality is found to be unacceptable, facility renovations might be the cause of disturbances to ceiling or wall structures, or as part of an investigation into a media fill or drug product sterility failure. Among the filters that should be leak tested are those installed in dry heat depyrogenation tunnels commonly used to depyrogenate glass vials.

Any aerosol used for challenging a HEPA filter should meet specifications for critical physicochemical attributes such as viscosity. Dioctylphthalate (DOP) and Poly-alpha-olefin (PAO) are examples of appropriate leak testing aerosols. Some alternative aerosols are problematic because they pose the risk of microbial contamination of the environment being tested. Firms should ensure that any alternative used does not promote microbial growth.

There is a major difference between *filter leak testing* and *efficiency testing*. An efficiency test is a general test used to determine only the rating of the filter.[7] An intact HEPA filter should be capable of retaining at least 99.97 percent of particulates greater than 0.3 micron in diameter.

The purpose of performing regularly scheduled leak tests, on the other hand, is to detect leaks from the filter media, filter frame, or seal. The challenge should be conducted using a polydispersed aerosol usually composed of particles with a light-scattering mean droplet diameter in the submicron size range, including a sufficient number of particles at approximately 0.3 microns. Performing a leak test without introducing a sufficient upstream challenge of particles of known size upstream of the filter is ineffective for detecting leaks. For example, depending on the accuracy of the photometer, a DOP challenge should introduce the aerosol upstream of the filter in a concentration ranging from approximately 25 to l00 micrograms/liter of air at the filter's designed airflow rating. The leak test should be done in place, and the filter face scanned on the downstream side with an appropriate photometer probe, at a sampling rate of at least one cubic foot per minute. The downstream leakage measured by the probe should then be calculated as a percent of the upstream challenge. Scanning should be conducted on the entire filter face and frame at a position about one to two inches from the face of the filter. This comprehensive scanning of HEPA filters should be fully documented.

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[6] The same broad principles can be applied to ULPA filters.

[7] The efficiency test uses a monodispersed aerosol of 0.3 micron size particles and assesses filter media. Downstream readings represent an average over the entire filter surface. Efficiency tests are not intended to test for filter leaks.

A single probe reading equivalent to 0.01 percent of the upstream challenge should be considered as indicative of a significant leak and should result in replacement of the HEPA filter or, when appropriate, repair in a limited area. A subsequent confirmatory re-test should be performed in the area of any repair.

HEPA filter leak testing alone is not sufficient to monitor filter performance. This testing is usually done only on a semi-annual basis. It is important to conduct periodic monitoring of filter attributes such as uniformity of velocity across the filter (and relative to adjacent filters). Variations in velocity generally increase the possibility of contamination, as these changes (e.g., velocity reduction) can have an effect on unidirectional airflow. Airflow velocities are measured 6 inches from the filter face and at a defined distance proximal to the work surface for HEPA filters in the critical area. Regular velocity monitoring can provide useful data on the clean area in which aseptic processing is performed. HEPA filters should be replaced when nonuniformity of air velocity across an area of the filter is detected or airflow patterns may be adversely affected.

Although vendors often provide these services, drug manufacturers are responsible for ensuring that these essential certification activities are conducted satisfactorily.

E. Design

21 CFR 211.42(b) states, in part, that "The flow of components, drug product containers, closures, labeling, in-process materials, and drug products through the building or buildings shall be designed to prevent contamination."

21 CFR 211.42(c) states, in part, that "There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: (i) Floors, walls, and ceilings of smooth, hard surfaces that are easily cleanable; *** (iii) An air supply filtered through high-efficiency particulate air filters under positive pressure *** (iv) A system for monitoring environmental conditions; (v) A system for cleaning and disinfecting the room and equipment to produce aseptic conditions; (vi) A system for maintaining any equipment used to control the aseptic conditions."

21 CFR 211.46(b) states that "Equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing, or holding of a drug product."

21 CFR 211.46(c) states, in part, that "Air filtration systems, including pre-filters and particulate matter air filters, shall be used when appropriate on air supplies to production areas."

21 CFR 211.63 states that "Equipment used in the manufacture, processing, packing, or holding of a drug product shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

21 CFR 211.65(a) states that "Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.67(a) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identify, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

Aseptic processes are designed to minimize exposure of sterile articles to the potential contamination hazards of the manufacturing operation. Limiting the duration of exposure of sterile product elements, providing the highest possible environmental control, optimizing process flow, and designing equipment to prevent entrainment of lower quality air into the Class 100 (ISO 5) clean area are essential to achieving high assurance of sterilty (Ref. 4).

Both personnel and material flow should be optimized to prevent unnecessary activities that could increase the potential for introducing contaminants to exposed product, container-closures, or the surrounding environment. The layout of equipment should provide for ergonomics that optimize comfort and movement of operators. The number of personnel in an aseptic processing room should be minimized. The flow of personnel should be designed to limit the frequency with which entries and exits are made to and from an aseptic processing room and, most significantly, its critical area. Regarding the latter, the number of transfers into an isolator, or into the critical area of a traditional clean room, should be minimized. To prevent changes in air currents that introduce lower quality air, movement adjacent to the critical area should be appropriately restricted.

Any intervention or stoppage during an aseptic process can increase the risk of contamination. The design of equipment used in aseptic processing should limit the number and complexity of aseptic interventions by personnel. For example, personnel intervention can be reduced by integrating an on-line weight check device, thus eliminating a repeated manual activity within the critical area. Rather than performing an aseptic connection, sterilizing the prefastened connection using sterilize-in-place (SIP) technology also can eliminate a significant aseptic manipulation. Automation of other process steps, including the use of technologies such as robotics, can further reduce risk to the product.

Transfer of products should be performed under appropriate cleanroom conditions. For example, lyophilization processes include transfer of aseptically filled product in partially sealed containers. To prevent contamination, partially closed sterile product should be transferred only in critical areas. Facility design should ensure that the area between a filling line and the lyophilizer and the transport and loading procedures provide Class 100 (ISO 5) protection.

The sterile drug product and container closures should be protected by equipment of suitable design. Carefully designed curtains, rigid plastic shields, or other barriers should be used in appropriate locations to achieve significant segregation of the aseptic processing line. Use of an isolator system further enhances product protection (see Appendix 1).

Due to the interdependence of the various rooms that make up an aseptic processing facility, it is essential to carefully define and control the dynamic interactions permitted between cleanrooms. Use of a double-door or integrated sterilizer is valuable in ensuring direct product flow, often from a lower to a higher classified area. Airlocks and interlocking doors facilitate better control of air balance throughout the aseptic processing facility. Airlocks should be installed between the aseptic processing area entrance and the adjoining uncontrolled area. Other interfaces such as personnel transitions or material staging areas are appropriate locations for air locks. It is critical to adequately control material (e.g., in-process supplies, equipment, utensils) as it transfers from lesser to higher controlled clean areas to prevent the influx of contaminants. For example, written procedures should address how materials should be introduced into the aseptic processing room to ensure that room conditions are not compromised. In this regard, materials should be disinfected in accord with appropriate procedures.

Cleanrooms are normally designed as functional units with specific purposes. A well-designed cleanroom is constructed with materials that allow for ease of cleaning and sanitizing. Examples of adequate design features include seamless and rounded floor to wall junctions as well as readily accessible corners. Floors, walls, and ceilings are constructed of smooth, hard surfaces that can be easily cleaned (211.42). Ceilings and associated HEPA filter banks should be designed to protect sterile materials from contamination. Cleanrooms also should not contain unnecessary equipment, fixtures, or materials.

Processing equipment and systems should be equipped with sanitary fittings and valves. With rare exceptions, drains are not considered appropriate for classified areas of the aseptic processing facility.

When applicable, equipment should be suitably designed for ease of sterilization (211.63). Ease of installation to facilitate aseptic setup is also an important consideration. The effect of equipment design on the cleanroom environment should be addressed. Flat surfaces or ledges that accumulate particles should be avoided. Equipment should not obstruct airflow and, in critical areas, its design should not perturb airflow.

Deviation or change control systems should address atypical conditions posed by shutdown of air handling systems or other utilities, and the impact of construction activities on facility control.

V. PERSONNEL TRAINING, QUALIFICATION, & MONITORING

21 CFR 211.22(c) states that "The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product."

21 CFR 211.25(a) states that "Each person engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training, and experience, or any combination thereof, to enable that person to perform the assigned functions. Training shall be in the particular operations that the employee performs and in current good manufacturing practice (including the current good manufacturing practice regulations in this chapter and written procedures required by these regulations) as they relate to the employee's functions. Training in current good manufacturing practice shall be conducted by qualified individuals on a continuing basis and with sufficient frequency to assure that employees remain familiar with CGMP requirements applicable to them."

21 CFR 211.25(b) states that "Each person responsible for supervising the manufacture, processing, packing, or holding of a drug product shall have the education, training, and experience, or any combination thereof, to perform assigned functions in such a manner as to provide assurance that the drug product has the safety, identity, strength, quality, and purity that it purports or is represented to possess."

21 CFR 211.25(c) states that "There shall be an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing, or holding of each drug product."

21 CFR 211.28(a) states that "Personnel engaged in the manufacture, processing, packing, or holding of a drug product shall wear clean clothing appropriate for the duties they perform. Protective apparel, such as head, face, hand, and arm coverings, shall be worn as necessary to protect drug products from contamination."

21 CFR 211.28(b) states that "Personnel shall practice good sanitation and health habits."

21 CFR 211.28(c) states that "Only personnel authorized by supervisory personnel shall enter those areas of the buildings and facilities designated as limited-access areas."

21 CFR 211.28(d) states that "Any person shown at any time (either by medical examination or supervisory observation) to have an apparent illness or open lesions that may adversely affect the safety or quality of drug products shall be excluded from direct contact with components, drug product containers, closures, in-process materials, and drug products until the condition is corrected or determined by competent medical personnel not to jeopardize the safety or quality of drug products. All personnel shall be instructed to report to supervisory personnel any health conditions that may have an adverse effect on drug products."

21 CFR 211.42(c) states, in part, that "There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: *** (iv) A system for monitoring environmental conditions***."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

A. Personnel

A well-designed aseptic process minimizes personnel intervention. As operator activities increase in an aseptic processing operation, the risk to finished product sterility also increases.

To ensure maintenance of product sterility, operators involved in aseptic manipulations should adhere to the basic principles of aseptic technique at all times.

Appropriate training should be conducted before an individual is permitted to enter the aseptic processing area and perform operations. For example, such training should include aseptic technique, cleanroom behavior, microbiology, hygiene, gowning, patient safety hazards posed by a nonsterile drug product, and the specific written procedures covering aseptic processing area operations. After initial training, personnel should be updated regularly by an ongoing training program. Supervisory personnel should routinely evaluate each operator's conformance to written procedures during actual operations. Similarly, the quality control unit should provide regular oversight of adherence to established, written procedures and basic aseptic techniques during manufacturing operations.

Some of these techniques aimed at maintaining sterility of sterile items and surfaces include:

- Contacting sterile materials only with sterile instruments

  Sterile instruments (e.g., forceps) should always be used in the handling of sterilized materials. Between uses, instruments should be placed only in sterilized containers. Instruments should be replaced as necessary throughout an operation.

  After initial gowning, sterile gloves should be regularly sanitized to minimize the risk of contamination. Personnel should not directly contact sterile products, containers, closures, or critical surfaces.

- Moving slowly and deliberately

  Rapid movements can create unacceptable turbulence in the critical zone. Such movements disrupt the sterile field, presenting a challenge beyond intended cleanroom design and control parameters. The principle of slow, careful movement should be followed throughout the cleanroom.

- Keeping the entire body out of the path of unidirectional air

  Unidirectional airflow design is used to protect sterile equipment surfaces, container-closures, and product. Personnel should not disrupt the path of unidirectional flow air in the aseptic processing zone.

- Approaching a necessary manipulation in a manner that does not compromise sterility of the product

  To maintain sterility of nearby sterile materials, a proper aseptic manipulation should be approached from the side and not above the product (in vertical unidirectional flow operations). Also, an operator should refrain from speaking when in direct proximity to an aseptic processing line.

- Maintaining Proper Gown Control

  Prior to and throughout aseptic operations, an operator should not engage in any activity that poses an unreasonable contamination risk to the gown.

Only personnel who have been qualified and appropriately gowned should be permitted access to the aseptic processing area. An aseptic processing area gown should provide a barrier between the body and exposed sterilized materials and prevent contamination from particles generated by, and microorganisms shed from, the body. Gowns should be sterile and nonshedding and should cover the skin and hair (face-masks, hoods, beard/moustache covers, protective goggles, elastic gloves, cleanroom boots, and shoe overcovers are examples of common elements of gowns). Written procedures should detail the methods used to don each gown component in an aseptic manner. An adequate barrier should be created by the overlapping of gown components (e.g., gloves overlapping sleeves). If an element of a gown is found to be torn or defective, it should be changed immediately.

There should be an established program to regularly assess or audit conformance of personnel to relevant aseptic manufacturing requirements. An aseptic gowning qualification program should assess the ability of a cleanroom operator to maintain the quality of the gown after performance of gowning procedures. Gowning qualification should include microbiological surface sampling of several locations on a gown (e.g., glove fingers, facemask, forearm, chest, other sites). Following an initial assessment of gowning, periodic requalification should monitor various gowning locations over a suitable period to ensure the consistent acceptability of aseptic gowning techniques. Semi-annual or yearly requalification is sufficient for automated operations where personnel involvement is minimized.

To protect exposed sterilized product, personnel should be expected to maintain gown quality and strictly adhere to appropriate aseptic method. Written procedures should adequately address circumstances under which personnel should be retrained, requalified, or reassigned to other areas.

B. Laboratory Personnel

The basic principles of training, aseptic technique, and personnel qualification in aseptic manufacturing also are applicable to those performing aseptic sampling and microbiological laboratory analyses. Processes and systems cannot be considered to be in control and reproducible if the validity of data produced by the laboratory is in question.

C. Monitoring Program

Personnel can significantly affect the quality of the environment in which the sterile product is processed. A vigilant and responsive personnel monitoring program should be established. Monitoring should be accomplished by obtaining surface samples of each operator's gloves on a daily basis, or in association with each batch. This sampling should be accompanied by an appropriate sampling frequency for other strategically selected locations of the gown (Ref. 5). The quality control unit should establish a more comprehensive monitoring program for operators involved in operations which are especially labor intensive (i.e., those requiring repeated or complex aseptic manipulations).

Asepsis is fundamental to an aseptic processing operation. An ongoing goal for manufacturing personnel in the aseptic processing room is to maintain contamination-free gloves throughout operations. Sanitizing gloves just prior to sampling is inappropriate because it can prevent recovery of microorganisms that were present during an aseptic manipulation. When operators exceed established levels or show an adverse trend, an investigation should be conducted promptly. Follow-up actions can include increased sampling, increased observation, retraining, gowning requalification, and in certain instances, reassignment of the individual to operations outside of the aseptic processing area. Microbiological trending systems, and assessment of the impact of atypical trends, are discussed in more detail under Section XI. Laboratory Controls.

VI. COMPONENTS AND CONTAINER/CLOSURES

A. Components

> 21 CFR 210.3(b)(3) states that "*Component* means any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product."
>
> 21 CFR 211.80(a) states that "There shall be written procedures describing in sufficient detail the receipt, identification, storage, handling, sampling, testing, and approval or rejection of components and drug product containers and closures; such written procedures shall be followed."
>
> 21 CFR 211.80(b) states that "Components and drug product containers and closures shall at all times be handled and stored in a manner to prevent contamination."
>
> 21 CFR 211.84(d)(6) states that "Each lot of a component, drug product container, or closure that is liable to microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use."

A drug product produced by aseptic processing can become contaminated through the use of one or more components (e.g., active ingredients, excipients, Water for Injection) that are contaminated with microorganisms or endotoxins. It is important to characterize the microbial content of each component that could be contaminated and establish appropriate acceptance limits based on information on bioburden. Knowledge of bioburden is critical in assessing whether the sterilization process is adequate.

In aseptic processing, each component is individually sterilized or several components are combined, with the resulting mixture sterilized.[8] There are several methods for sterilizing components (see relevant discussion in Section IX). A widely used method is filtration of a solution formed by dissolving the component(s) in a solvent such as USP Water for Injection (WFI). The solution is passed through a sterilizing membrane or cartridge filter. Filter sterilization is used where the component is soluble and is likely to be adversely affected by heat. A variation of this method involves subjecting the filtered solution to aseptic crystallization and precipitation (or lyophilization) of the component as a sterile powder. However, this method involves more handling and manipulation and therefore has a higher potential for contamination during processing. If a component is not adversely affected by heat, and is soluble, it can be made into a solution and subjected to steam sterilization, typically in an autoclave or a fixed pressurized sterilize-in-place (SIP) vessel.

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[8] See Appendix III for discussion of certain biologic components that are aseptically handled from the start of the process.

Dry heat sterilization is a suitable method for components that are heat stable and insoluble. However, carefully designed heat penetration and distribution studies should be performed for powder sterilization because of the insulating effects of the powder.

Ethylene oxide (EtO) exposure is often used for surface sterilization, and for sterilizing certain packages with porous overwrapping. Such methods should be carefully controlled and validated if used for powders to evaluate whether consistent penetration of the sterilant can be achieved and to minimize residual ethylene oxide and by-products.

Parenteral products are intended to be nonpyrogenic. There should be written procedures and appropriate specifications for acceptance or rejection of each lot of components that might contain endotoxins. Any components failing to meet defined endotoxin limits should be rejected.

B. Containers/Closures

21 CFR 211.94(c) states that "Drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use."

21 CFR 211.94(d) states that "Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing, and processing to remove pyrogenic properties shall be written and followed for drug product containers and closures."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

1. Preparation

Containers and closures should be rendered sterile and, for parenteral drug products, pyrogen-free. The type of processes used will depend primarily on the nature of the container and/or closure materials. The validation study for such a process should be adequate to demonstrate its ability to render materials sterile and pyrogen-free. Written procedures should specify the frequency of revalidation of these processes as well as time limits for holding sterile, depyrogenated containers and closures.

Presterilization preparation of glass containers usually involves a series of wash and rinse cycles. These cycles serve an important role in removing foreign matter. Rinse water should be of high purity so as not to contaminate containers. For parenteral products, final rinse water should meet the specifications of Water for Injection, USP.

The adequacy of the depyrogenation process can be assessed by spiking containers or closures with known quantities of endotoxin, followed by measuring endotoxin content after depyrogenation. The challenge studies should be performed with a reconstituted endotoxin solution applied directly onto the surface being tested and air-dried. Positive controls should be used to measure the percentage of endotoxin recovery by the test method. Validation study data should demonstrate that the process reduces the endotoxin content by at least 99.9 percent (3 logs) (see Section VII).

Glass containers are generally subjected to dry heat for sterilization and depyrogenation. Validation of dry heat sterilization and depyrogenation should include appropriate heat distribution and penetration studies as well as the use of worst-case process cycles, container characteristics (e.g., mass), and specific loading configurations to represent actual production runs. See Section IX.C.

Pyrogen on plastic containers can be generally removed by multiple WFI rinses. Plastic containers can be sterilized with an appropriate gas, irradiation, or other suitable means. For gases such as EtO, the parameters and limits of the EtO sterilization cycle (e.g. temperature, pressure, humidity, gas concentration, exposure time, degassing, aeration, and determination of residuals) should be specified and monitored closely. Biological indicators are of special importance in demonstrating the effectiveness of EtO and other gas sterilization processes.

Rubber closures (e.g., stoppers and syringe plungers) can be cleaned by multiple cycles of washing and rinsing prior to final steam or irradiation sterilization. At minimum, the initial rinses for the washing process should employ Purified Water, USP, of minimal endotoxin content, followed by final rinse(s) with WFI for parenteral products. Normally, depyrogenation is achieved by multiple rinses of hot WFI. The time between washing, drying (where appropriate), and sterilizing should be minimized because residual moisture on the stoppers can support microbial growth and the generation of endotoxins. Because rubber is a poor conductor of heat, extra attention should be given to the validation of processes that use heat with respect to its penetration into the rubber stopper load (See Section XI.C). Validation data from the washing procedure should demonstrate successful endotoxin removal from rubber materials.

A potential source of contamination is the siliconization of rubber stoppers. Silicone used in the preparation of rubber stoppers should meet appropriate quality control criteria and not have an adverse effect on the safety, quality, or purity of the drug product.

Contract facilities that perform sterilization and/or depyrogenation of containers and closures are subject to the same CGMP requirements as those established for in-house processing. The finished dosage form manufacturer is responsible for the review and approval of the contractor's validation protocol and final validation report.

2. Inspection of Container Closure System

A container closure system that permits penetration of air, or microorganisms, is unsuitable for a sterile product. Any damaged or defective units should be detected, and removed, during inspection of the final sealed product. Safeguards should be implemented to strictly preclude shipment of product that may lack container closure integrity and lead to nonsterility. Equipment suitability problems or incoming container or closure deficiencies have caused loss of container closure system integrity. As examples, failure to detect vials fractured by faulty machinery, or by mishandling of bulk finished stock, has led to drug recalls. If damage that is not readily detected leads to loss of container closure integrity, improved procedures should be rapidly implemented to prevent and detect such defects.

Functional defects in delivery devices (e.g., syringe device defects, delivery volume) can also result in product quality problems and should be monitored by appropriate in-process testing.

Any defects or results outside the specifications established for in-process and final inspection should be investigated in accord with Section 211.192.

VII. ENDOTOXIN CONTROL

21 CFR 211.63 states that "Equipment used in the manufacture, processing, packing, or holding of a drug product shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

21 CFR 211.65(a) states that "Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.67(a) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identify, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.94(c) states that "Drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use."

21 CFR 211.167(a) states that "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

Endotoxin contamination of an injectable product can be a result of poor CGMP controls. Certain patient populations (e.g., neonates), those receiving other injections concomitantly, or those administered a parenteral in atypically large volumes or doses can be at greater risk for pyrogenic reaction than anticipated by the established limits based on body weight of a normal healthy adult (Ref. 6, 7). Such clinical concerns reinforce the need for appropriate CGMP controls to prevent generation of endotoxin. Drug product components, container closures, equipment, and storage time limitations are among the areas to address in establishing endotoxin control.

Adequate cleaning, drying, and storage of equipment provides for control of bioburden and prevents contribution of endotoxin load. Equipment should be designed to be easily assembled and disassembled, cleaned, sanitized, and/or sterilized. Endotoxin control should be exercised for all product contact surfaces both prior to and after sterile filtration.

Endotoxin on equipment surfaces is inactivated by high-temperature dry heat, or removed from equipment surfaces by validated cleaning procedures. Some clean-in-place procedures employ initial rinses with appropriate high purity water and/or a cleaning agent (e.g., acid, base, surfactant), followed by final rinses with heated WFI. Equipment should be dried following cleaning. Sterilizing-grade filters and moist heat sterilization have not been shown to be effective in removing endotoxins. Processes that are designed to achieve depyrogenation should demonstrate a 3-log reduction of endotoxin.

VIII. TIME LIMITATIONS

> 21 CFR 211.111 states, in part, that "When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product."

Time limits should be established for each phase of aseptic processing. Time limits should include, for example, the period between the start of bulk product compounding and its filtration, filtration processes, product exposure while on the processing line, and storage of sterilized equipment, containers and closures. Maintenance of in-process quality at different production phases should be supported by data. Bioburden and endotoxin load should be assessed when establishing time limits for stages such as the formulation processing stage.

The total time for product filtration should be limited to an established maximum to prevent microorganisms from penetrating the filter. Such a time limit should also prevent a significant increase in upstream bioburden and endotoxin load. Sterilizing-grade filters should generally be replaced following each manufactured lot. Because they can provide a substrate for microbial attachment, maximum use times for those filters used upstream for solution clarification or particle removal should also be established and justified.

IX. VALIDATION OF ASEPTIC PROCESSING AND STERILIZATION

> 21 CFR 211.63, 211.65, and 211.67 address, respectively, "Equipment design, size, and location," "Equipment construction," and "Equipment cleaning and maintenance."
>
> 21 CFR 211.84(c)(3) states that "Sterile equipment and aseptic sampling techniques shall be used when necessary."
>
> 21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

This section primarily discusses routine qualification and validation study recommendations. Change control procedures are addressed only briefly, but are an important part of the quality systems established by a firm. As noted above, a change in equipment, process, test method, or systems should be evaluated through the written change control program and should trigger an evaluation of the need for revalidation or requalification.

A. Process Simulations

To ensure the sterility of products purporting to be sterile, both sterilization and aseptic filling and closing operations must be adequately validated (211.113). The goal of even the most effective sterilization processes can be defeated if the sterilized elements of a product (the drug, the container, and the closure) are brought together under conditions that contaminate any of those elements. Similarly, product sterility will be compromised if product elements are not sterile when they are assembled.

The validation of an aseptic processing operation should include the use of a microbiological growth nutrient medium in place of the product. This has been termed a *media fill or process simulation*. In the normal media fill simulation, the nutrient medium should be exposed to product contact surfaces of equipment, container closure systems, critical environments, and process manipulations to closely simulate the same exposure that the product itself will undergo. The sealed containers filled with the media are then incubated to detect microbial contamination. The results should be interpreted to determine the potential for a unit of drug product to become contaminated during actual operations (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing). Environmental monitoring data from the process simulation can also provide useful information for the processing line evaluation.

1. Study Design

A recommended media fill program incorporates the contamination risk factors that occur on a production line, and accurately assesses the state of process control. Media fill studies should simulate aseptic manufacturing operations as closely as possible, incorporating a worst-case approach. The media fill program should address applicable issues such as:

- factors associated with the longest permitted run on the processing line
- number and type of normal interventions, atypical interventions, unexpected events (e.g., maintenance), stoppages, equipment adjustments or transfers
- lyophilization, when applicable
- aseptic assembly of equipment (e.g., at start-up, during processing)
- number of personnel and their activities
- number of aseptic additions (e.g., charging containers and closures as well as sterile ingredients)
- shift changes, breaks, and gown changes (when applicable)
- number and type of aseptic equipment disconnections/connections
- aseptic sample collections
- line speed and configurations
- manual weight checks
- operator fatigue
- container closure systems (e.g., sizes, type, compatibility with equipment)
- specific provisions of aseptic processing related Standard Operating Procedures (e.g., conditions permitted before line clearance is mandated)

A written batch record, documenting production conditions and simulated activities, should be prepared for each media fill run. The same vigilance should be observed in both media fill and routine production runs. Media fills should not be used to justify an unacceptable practice.

2. Frequency and Number of Runs

When a processing line is initially qualified, separate media fills should be repeated enough times to ensure that results are consistent and meaningful. This approach is important because a single run can be inconclusive, while multiple runs with divergent results signal a process that is not in control. At least three consecutive separate successful runs should be performed during initial line qualification. Subsequently, routine semi-annual qualification should be conducted for each processing line to evaluate the state of control of the aseptic process. Activities and interventions representative of each shift, and shift changeover, should be incorporated into the design of the semi-annual qualification. For example, the evaluation of a shift should address its unique time-related and operational features. All personnel who enter the aseptic processing area, including technicians and maintenance personnel, should participate in a media fill at least once a year. Participation should be consistent with the nature of each operator’s duties during routine production. Each change to a product or line change should be evaluated using a written change control system. Any changes or events that have the potential to affect the ability of the aseptic process to exclude contamination from the sterilized product should be assessed through additional media fills. For example, facility and equipment modifications, line configuration changes, significant changes in personnel, anomalies in environmental testing results, container closure system changes or, end product sterility testing showing contaminated products may be cause for revalidation of the system.

Where data from a media fill indicate the process may not be in control, a comprehensive documented investigation should be conducted to determine the origin of the contamination and the scope of the problem. Once corrections are instituted, repeat process simulation runs should be performed to confirm that deficiencies in practices and procedures have been corrected and the process has returned to a state of control. When an investigation fails to reach well-supported, substantive conclusions as to the cause of the media fill failure, three consecutive successful runs and increased scrutiny (e.g., extra supervision, monitoring) of the production process should be implemented.

3. Duration of Runs

The duration of aseptic processing operations is a major consideration in determining the size of the media fill run. Although the most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production run, other appropriate models can be justified. In any study protocol, the duration of the run and the overall study design should adequately mimic worst-case operating conditions and cover all manipulations that are performed in the actual processing operation. In this regard, interventions that commonly occur should be routinely simulated, while those occurring rarely can be simulated periodically.

While conventional manufacturing lines are highly automated, often operate at relatively high speeds, and are designed to limit operator intervention, there are some processes that include considerable operator involvement. When aseptic processing employs manual filling or closing, or extensive manual manipulations, the duration of the process simulation should generally be no less than the length of the actual manufacturing process to best simulate contamination risks posed by operators.

For lyophilization operations, unsealed containers should be exposed to pressurization and partial evacuation of the chamber in a manner that simulates the process. Vials should not be frozen, as this may inhibit the growth of microorganisms.

4. Size of Runs

The simulation run sizes should be adequate to mimic commercial production conditions and accurately assess the potential for commercial batch contamination. The number of units filled during the process simulation should be based on contamination risk for a given process and sufficient to accurately simulate activities that are representative of the manufacturing process. A generally acceptable starting point for run size is in the range of 5,000 to 10,000 units. For operations with production sizes under 5,000, the number of media filled units should equal the maximum batch size made on the processing line (Ref. 8).

When the possibility of contamination is higher based on the process design (e.g., manually intensive filling lines), a larger number of units, generally at or approaching the full production batch size, should be used. In contrast, a process conducted in an isolator (see Appendix 1) can have a low risk of contamination because of the lack of direct human intervention and can be simulated with a lower number of units as a proportion of the overall operation.

Some batches are produced over multiple shifts or yield an unusually large number of units, and media fill size and duration are especially important considerations in the media fill protocol. These factors should be carefully considered when designing the simulation to adequately encompass conditions and any potential risks associated with the larger operation.

5. Line Speed

The media fill program should adequately address the range of line speeds (e.g., by bracketing all vial sizes and fill volumes) employed during production. Each individual media fill run should evaluate a single worst-case line speed, and the speed chosen for each run during a study should be justified. For example, use of high line speed is often most appropriate in the evaluation of manufacturing processes characterized by frequent interventions or a significant degree of manual manipulation. Use of slow line speed is generally appropriate for evaluating manufacturing processes characterized by prolonged exposure of the sterile drug product and container closures in the aseptic area.

6. Environmental Conditions

Media fills should be adequately representative of the range of conditions under which actual manufacturing operations are conducted. An inaccurate assessment (making the process appear cleaner than it actually is) can result from conducting a media fill under extraordinary air particulate and microbial quality, or under production controls and precautions taken in preparation for the media fill. To the extent standard operating procedures permit stressful conditions, it is important that media fills include analogous challenges to support the validity of these studies.

7. Media

In general, a microbiological growth medium, such as soybean casein digest medium, should be used. Use of anaerobic growth media (e.g., fluid thioglycollate medium) would be appropriate in special circumstances. The media selected should be demonstrated to promote growth of USP <71> indicator microorganisms as well as representative isolates identified by environmental monitoring, personnel monitoring, and positive sterility test results. Positive control units should be inoculated with a <100 CFU challenge and incubated. For those instances in which the growth promotion testing fails, the origin of any contamination found during the simulation should nonetheless be investigated, and the media fill should be promptly repeated.

The production process should be accurately simulated using media and conditions that optimize detection of any microbiological contamination. Each unit should be filled with an appropriate quantity and type of microbial growth medium to contact the inner container closure surfaces (when the unit is inverted or thoroughly swirled) and permit visual detection of microbial growth.

Some drug manufacturers have expressed concern over the possible contamination of the facility and equipment with the nutrient media during media fill runs. However, if the medium is handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary, sterilization of equipment, subsequently processed products are not likely to be compromised.

8. Incubation and Examination of Media-Filled Units

Media units should be incubated under conditions adequate to detect organisms that can otherwise be difficult to culture. Incubation conditions should be established in accord with the following general guidelines:

- Incubation temperature should be suitable for recovery of bioburden and environmental isolates and should at no time be outside the range of 20-35°C. Incubation temperature should be maintained within 2.5^{o}C of the target temperature.

- Incubation time should not be less than 14 days. If two temperatures are used for the incubation of the media filled samples, the samples should be incubated for at least 7 days at each temperature.

Each media-filled unit should be examined for contamination by personnel with appropriate education, training, and experience in microbiological techniques. There should be direct quality control unit oversight throughout any such examination. Clear containers with otherwise identical physical properties should be used as a substitute for amber or other opaque containers to allow visual detection of microbial growth.

When a firm performs a final product inspection of units immediately following the media fill run, all integral units should proceed to incubation. Units found to have defects not related to integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be rejected. Erroneously rejected units should be returned promptly for incubation with the media fill lot.

After incubation is underway, any unit found to be damaged should be included in the data for the media fill run, because the incubation of the units simulates release to the market. Any decision to exclude such incubated units (i.e., nonintegral) from the final run tally should be fully justified and the deviation explained in the media fill report. If a correlation emerges between difficult to detect damage and microbial contamination, a thorough investigation should be conducted to determine its cause (see Section VI.B).

Written procedures regarding aseptic interventions should be clear and specific (e.g., intervention type; quantity of units removed), providing for consistent production practices and assessment of these practices during media fills. If written procedures and batch documentation are adequate, these intervention units do not need to be incubated during media fills.[9] Where procedures lack specificity, there would be insufficient justification for exclusion of units removed during an intervention from incubation. As an example, if a production procedure requires removal of 10 units after an intervention at the stoppering station infeed, batch records (i.e., for production and media fills) should clearly document conformance with this procedure. In no case should more units be removed during a media fill intervention than would be cleared during a production run. The ability of a media fill run to detect potential contamination from a given simulated activity should not be compromised by a large-scale line clearance, which can result in removal of a positive unit caused by an unrelated event or intervention. If unavoidable, appropriate study provisions should be made to compensate in such instances.

Appropriate criteria should be established for yield and accountability. Media fill record reconciliation documentation should include a full accounting and description of units rejected from a batch.

9. *Interpretation of Test Results*

The process simulation run should be observed, and contaminated units should be reconcilable with the approximate time and the activity being simulated during the media fill. Video recording of a media fill has been found to be useful in identifying personnel practices that could negatively impact the aseptic process.

Any contaminated unit should be considered as objectionable and fully investigated. The microorganisms should be identified to species level. In the case of a media fill failure, a comprehensive investigation should be conducted, surveying all possible causes of the contamination. The effects on commercial drugs produced on the line since the last successful media fill should also be assessed.

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[9] To assess contamination risk during initial aseptic setup (before fill), valuable information can be obtained by incubating all such units that may be normally removed.

Whenever contamination exists in a media fill run, it should be considered indicative of a potential sterility assurance problem, regardless of run size. The number of contaminated units should not be expected to increase in a directly proportional manner with the number of vials in the media fill run. Test results should reliably and reproducibly show that the units produced by an aseptic processing operation are sterile. Modern aseptic processing operations in suitably designed facilities have demonstrated a capability of meeting contamination levels approaching zero (Ref. 8, 9) and should normally yield no media fill contamination. Recommended criteria for assessing state of aseptic line control are as follows:

- When filling fewer than 5000 units, no contaminated units should be detected.

- When filling from 5,000 to 10,000 units:
  -- 1 contaminated unit should result in an investigation, including consideration of a repeat media fill.
  -- 2 contaminated units are considered cause for revalidation, following investigation.

- When filling more than 10,000 units:
  -- 1 contaminated unit should result in an investigation.
  -- 2 contaminated units are considered cause for revalidation, following investigation.

For any run size, intermittent incidents of microbial contamination in media filled runs can be indicative of a persistent low-level contamination problem that should be investigated. Accordingly, recurring incidents of contaminated units in media fills for an individual line, regardless of acceptance criteria, would be a signal of an adverse trend on the aseptic processing line that should lead to problem identification, correction, and revalidation.

A firm's use of media fill acceptance criteria allowing infrequent contamination does not mean that a distributed lot of drug product purporting to be sterile may contain a nonsterile unit. The purpose of an aseptic process is to prevent any contamination. A manufacturer is fully liable for the shipment of any nonsterile unit, an act that is prohibited under the FD&C Act (§ 301(a) 21 U.S.C. 331(a)). FDA also recognizes that there might be some scientific and technical limitations on how precisely and accurately validation can characterize a system of controls intended to exclude contamination.

As with any validation run, it is important to note that *invalidation* of a media fill run should be a rare occurrence. A media fill run should be aborted only under circumstances in which written procedures require commercial lots to be equally handled. Supporting documentation and justification should be provided in such cases.

B. Filtration Efficacy

Filtration is a common method of sterilizing drug product solutions. An appropriate sterilizing grade filter is one that reproducibly removes all microorganisms from the process stream, producing a sterile effluent. Such filters usually have a rated porosity of 0.2 micron or smaller. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate worst-case production conditions regarding the size of microorganisms in the material to be filtered and integrity test results of the filters used for the study. The microorganisms should be small enough to both challenge the nominal porosity of the filter and simulate the smallest microorganism that may occur in production. The microorganism *Brevundimonas diminuta* (ATCC 19146) when properly grown, harvested and used, can be satisfactory in this regard because it is one of the smallest bacteria (0.3 micron mean diameter). Bioburden of unsterilized bulk solutions should be determined to trend the characteristics of potentially contaminating organisms. In certain cases, when justified as equivalent or better than use of *Brevundimonas diminuta*, it may be appropriate to conduct bacterial retention studies with a bioburden isolate. The number of microorganisms in the challenge is important because a filter can contain a number of pores larger than the nominal rating, which has the potential to allow passage of microorganisms. The probability of such passage is considered to increase as the number of organisms (bioburden) in the material to be filtered increases. A challenge concentration of at least 10^7 organisms per cm^2 of effective filtration area of *B. diminuta* should generally be used. A commercial lot's actual influent bioburden should not include microorganisms of a size and/or concentration that would present a challenge beyond that considered by the validation study (Refs. 10, 11, 12).

Direct inoculation into the drug formulation provides an assessment of the effect of drug product on the filter matrix and on the challenge organism. However, directly inoculating *B. diminuta* into products with inherent bactericidal activity or into oil-based formulations can lead to erroneous conclusions. When sufficiently justified, the effects of the product formulation on the membrane's integrity can be assessed using an appropriate alternate method. For example, the drug product could be filtered in a manner in which the worst-case combination of process specifications and conditions are simulated. This step could be followed by filtration of the challenge organism for a significant period of time, under the same conditions, using an appropriately modified product (e.g., lacking an antimicrobial preservative or other antimicrobial component) as the vehicle. Any divergence from a simulation using the actual product and conditions of processing should be justified.

Factors that can affect filter performance normally include (1) viscosity of the material to be filtered, (2) pH, (3) compatibility of the material or formulation components with the filter itself, (4) pressures, (5) flow rates, (6) maximum use time, (7) temperature, (8) osmolality, (9) and the effects of hydraulic shock. When designing the validation protocol, it is important to address the effect of the extremes of processing factors on the filter capability to produce sterile effluent. Filter validation should be conducted using the worst-case conditions, such as maximum filter use time and pressure (Ref. 12). Filter validation experiments, including microbial challenges, need not be conducted in the actual manufacturing areas. However, it is essential that laboratory experiments simulate actual production conditions. The specific type of filter used in commercial production should be evaluated in filter validation studies. When the more complex filter validation tests go beyond the capabilities of the filter user, tests are often conducted by outside laboratories or by filter manufacturers. However, it is the responsibility of the filter user to review the validation data on the efficacy of the filter in producing a sterile effluent. The data should be applicable to the user's products and conditions of use because filter performance may differ significantly for various conditions and products.

After a filtration process is properly validated for a given product, process, and filter, it is important to ensure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. Sterilizing filters should be routinely discarded after processing of a single batch. Normally, integrity testing of the filter is performed prior to processing, after the filter apparatus has already been assembled and sterilized. It is important that integrity testing be conducted after filtration to detect any filter leaks or perforations that might have occurred during the filtration. *Forward flow and bubble point* tests, when appropriately employed, are two integrity tests that can be used. A production filter's integrity test specification should be consistent with data generated during filtration efficacy studies.

We recommend you consider use of sterilizing-grade filters in series; this is a common practice.

C. Sterilization of Equipment and Container and Closures

To maintain sterility, equipment surfaces that contact a sterilized drug product or sterilized container or closure surfaces must be sterile so as not to alter purity of the drug (211.63 and 211.113). Those surfaces that are in the vicinity of sterile product or container closures, but do not directly contact the product should also be rendered sterile where reasonable contamination potential exists. It is as important in aseptic processing to properly validate the processes used to sterilize such critical equipment as it is to validate processes used to sterilize the drug product and its container and closure. Moist heat and dry heat sterilization are most widely used and the primary processes discussed in this document. It should be noted that many of the heat sterilization principles discussed in this document are also applicable to other sterilization methods.

Sterility of aseptic processing equipment should be maintained by batch-by-batch sterilization. Following sterilization of equipment, containers, or closures, transportation or assembly should be performed with adherence to strict aseptic methods in a manner that protects and sustains the product's sterile state.

1. Sterilizer Qualification and Validation

Validation studies should be conducted demonstrating the efficacy of the sterilization cycle. Requalification studies should also be performed on a periodic basis. For both the validation studies and routine production, use of a specified load configuration should be documented in the batch records.

The insulating properties of unevacuated air prevent moist heat under pressure from penetrating or heating up materials and achieving the lethality associated with saturated steam. Consequently, for such processes, there is a far slower thermal energy transfer and rate of kill from the dry heat in insulated locations in the load. It is important to remove air from the autoclave chamber as part of a moist heat under pressure sterilization cycle.

For the various methods of sterilization, special attention should be given to the nature or type of the materials to be sterilized and the placement of biological indicators within the sterilization load. D-value of the biological indicator can vary widely depending on the material to be sterilized. Potentially difficult to reach locations within the sterilizer load or equipment train (for SIP applications) should be evaluated in initial studies. For example, filter installations in piping can cause a substantial pressure differential across the filter, resulting in a significant temperature drop on the downstream side. Biological indicators should be placed at appropriate downstream locations of this equipment to determine if the drop in temperature affects the thermal input at these sites. Requalification and/or revalidation should continue to focus on the load areas identified as most difficult to penetrate or heat (e.g., worst-case locations of tightly wrapped or densely packed supplies, securely fastened load articles, lengthy tubing, the sterile filter apparatus, hydrophobic filters, stopper load).

The formal program providing for regular revalidation should consider the age of the sterilizer and its past performance. Change control procedures should adequately address issues such as a load configuration change or a modification of the sterilizer.

a. Qualification: Empty Chamber

Temperature distribution studies evaluate numerous locations throughout an empty sterilizing unit (e.g., steam autoclave, dry heat oven) or equipment train (e.g., large tanks, immobile piping). It is important that these studies assess temperature uniformity at various locations throughout the sterilizer to identify potential *cold spots* where there can be insufficient heat to attain sterility. These heat uniformity or *temperature mapping* studies should be conducted by placing calibrated temperature measurement devices in numerous locations throughout the chamber.

b. Validation: Loaded Chamber

Heat penetration studies should be performed using the established sterilizer load(s). Validation of the sterilization process with a loaded chamber demonstrates the effects of loading on thermal input to the items being sterilized, and may identify *cold spots* where there is insufficient heat to attain sterility. The placement of biological indicators (BI) at numerous positions in the load, including the most difficult to sterilize places, is a direct means of demonstrating the efficacy of any sterilization procedure. In general, the thermocouple (TC) is placed adjacent to the BI so as to assess the correlation between microbial lethality and thermal input. When determining which articles are most difficult to sterilize, special attention should be given to the sterilization of filters.

Ultimately, cycle specifications for such sterilization methods are based on the delivery of adequate thermal input to the slowest to heat locations. A sterility assurance level of 10^{-6} or better should be demonstrated for a sterilization process. For more information, please also refer to the FDA guidance entitled *Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products*.

2. Equipment Controls and Instrument Calibration

For both validation and routine process control, the reliability of the data generated by sterilization cycle monitoring devices should be considered to be of the utmost importance. Devices that measure cycle parameters should be routinely calibrated. Written procedures should be established to ensure that these devices are maintained in a calibrated state. For example:

- Temperature monitoring devices for heat sterilization should be calibrated at suitable intervals, as well as before and after validation runs.
- Devices used to monitor dwell time in the sterilizer should be periodically calibrated.
- The microbial count and D-value of a biological indicator should be confirmed before a validation study.
- Bacterial endotoxin challenges should be appropriately prepared and measured by the laboratory.
- Instruments used to determine the purity of steam should be calibrated as appropriate.
- For dry heat depyrogenation tunnels, devices (e.g. sensors and transmitters) used to measure belt speed should be routinely calibrated.

To ensure robust process control, sterilizing equipment should be properly designed with attention to features such as accessibility to sterilant, piping slope, and proper condensate removal (as applicable). Equipment control should be ensured through placement of measuring devices at those risk-based control points that are most likely to rapidly detect unexpected process variability. Where manual manipulations of valves are required for sterilizer operations, these steps should be documented in manufacturing procedures. Sterilizing equipment should be properly maintained to allow for consistently satisfactory function. Evaluation of sterilizer performance attributes such as equilibrium (come up) time studies should be helpful in assessing if the unit continues to operate properly.

X. LABORATORY CONTROLS

21 CFR 211.22(c) states that "The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product."

21 CFR 211.42(c) states, in part, that "There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: *** (10) Aseptic processing, which includes as appropriate: *** (iv) A system for monitoring environmental conditions***."

21 CFR 211.56(b) states that "There shall be written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities; such written procedures shall be followed."

21 CFR 211.56(c) states, in part, that "There shall be written procedures for use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents. Such written procedures shall be designed to prevent the contamination of equipment, components, drug product containers, closures, packaging, labeling materials, or drug products and shall be followed."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

21 CFR 211.160(b) states that "Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity. Laboratory controls shall include: (1) Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of components, drug product containers, closures, and labeling used in the manufacture, processing, packing, or holding of drug products. The specifications shall include a description of the sampling and testing procedures used. Samples shall be representative and adequately identified. Such procedures shall also require appropriate retesting of any component, drug product container, or closure that is subject to deterioration. (2) Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. Such samples shall be representative and properly identified. (3) Determination of conformance to written descriptions of sampling procedures and appropriate specifications for drug products. Such samples shall be representative and properly identified. (4) The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used."

21 CFR 211.165(e) states that "The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with 211.194(a)(2)."

21 CFR 211.192 states, in part, that "All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed."

A. Environmental Monitoring

1. General Written Program

In aseptic processing, one of the most important laboratory controls is the establishment of an environmental monitoring program. This monitoring provides meaningful information on the quality of the aseptic processing environment (when a given batch is being manufactured) as well as environmental trends of the manufacturing area. An adequate program identifies potential routes of contamination, allowing for implementation of corrections before product contamination occurs (211.42 and 211.113).

Evaluating the quality of air and surfaces in the cleanroom environment should start with a well-defined written program and scientifically sound methods. The monitoring program should cover all production shifts and include air, floors, walls, and equipment surfaces, including the critical surfaces that come in contact with the product, container, and closures. Written procedures should include a list of locations to be sampled. Sample timing, frequency, and location should be carefully selected based upon their relationship to the operation performed. Samples should be taken throughout the aseptic processing facility (e.g., aseptic corridors, gowning rooms) using scientifically sound sampling procedures. Sampling sizes should be sufficient to optimize detection of environmental contaminants at levels that might be expected in a given clean area.

Locations posing the most microbiological risk to the product are a critical part of the program. It is especially important to monitor the microbiological quality of the aseptic processing clean area to determine whether or not aseptic conditions are maintained during filling and closing activities. Air and surface samples should be taken at the actual working site and at locations where significant activity or product exposure occurs during production. Critical surfaces that come in contact with the sterile product should be sterile. When identifying critical sites to be sampled, consideration should be given to the points of contamination risk in a process, including factors such as difficulty of setup, length of processing time, impact of interventions. Critical surface sampling should be performed at the conclusion of the aseptic processing operation to avoid direct contact with sterile surfaces during processing. Detection of microbial contamination on a critical site should not necessarily result in batch rejection. The contaminated critical site sample should be investigated with an awareness of the potential for a low incidence of false positives and should include an assessment of operational information and data.

Environmental monitoring methods do not always recover microorganisms present in the sampled area. In particular, low-level contamination can be particularly difficult to detect. Because of the likelihood of false negatives, consecutive growth results are only one type of adverse trend. Increased incidence of contamination over a given period is an equal or more significant trend to be tracked.

In the absence of any adverse trend, a single result above an action level should trigger an evaluation and a determination about whether remedial measures may be appropriate. In all room classes, remedial measures should be taken in response to unfavorable trends.

All environmental monitoring locations should be described in SOPs with sufficient detail to allow for reproducible sampling of a given location surveyed. Written SOPs should also address areas such as (1) frequency of sampling, (2) when the samples are taken (i.e., during or at the conclusion of operations), (3) duration of sampling, (4) sample size (e.g., surface area, air volume), (5) specific sampling equipment and techniques, (6) alert and action levels, and (7) appropriate response to deviations from alert or action levels.

2. Establishing Levels and a Trending Program

Microbiological monitoring levels should be established based on the relationship of the sampled location to the operation. The levels should be based on the need to maintain adequate microbiological control throughout the entire sterile manufacturing facility. One should also consider environmental monitoring data from historical databases, media fills, cleanroom qualification, and sanitization studies, in developing monitoring levels. Published data from similar operations can also be helpful in setting action and alert levels, especially for a new operation.

Monitoring the microbiological quality of the environment should include both alert and action levels. Each individual sample result should be evaluated for its significance by comparison to the alert or action levels. Averaging of results can mask unacceptable localized conditions. A result at the alert level urges attention to the approaching action conditions. A result at the action level should prompt a more thorough investigation. Written procedures should be established, detailing data review frequency, identification of contaminants, and actions to be taken. The quality control unit should provide routine oversight of near-term (e.g., daily, weekly, monthly, quarterly) and long-term trends in environmental and personnel monitoring data.

Trend reports should include data generated by location, shift, lot, room, operator, or other search parameters. The quality control unit should be responsible for producing specialized data reports (e.g., a search on a particular isolate over a year period) with the goal of investigating results beyond established levels and identifying any appropriate follow-up actions. Significant changes in microbial flora should be considered in the review of the ongoing environmental monitoring data.

Written procedures should define the system whereby the most responsible managers are regularly informed and updated on trends and investigations.

3. Sanitization Efficacy

The suitability, efficacy, and limitations of sanitization agents and procedures should be assessed. The effectiveness of these sanitization agents and procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces (i.e., via obtaining samples before and after sanitization).

Upon preparation, disinfectants should be rendered sterile, and used for a limited time, as specified by written procedures. Routinely used disinfectants should be effective against the normal microbial vegetative flora recovered from the facility. Many common sanitizers are ineffective against spores, for example, 70 percent isopropyl alcohol is ineffective against *Bacillus*, spp. spores. Therefore a sound disinfectant program also includes a sporicidal agent, used according to a written schedule and when environmental data suggest the presence of sporeforming organisms.

Sanitization procedures should be described in sufficient detail (e.g., preparation, work sequence, contact time) to enable reproducibility. Once the procedures are established, their adequacy should be evaluated using a routine environmental monitoring program.

4. Monitoring Methods

Acceptable methods for monitoring the microbiological quality of the environment include:

a. Surface Monitoring

Environmental monitoring should include testing of various surfaces for microbiological quality. For example, product contact surfaces, floors, walls, ceilings, and equipment should be tested on a regular basis. Routinely used for such tests are touch plates, swabs, and contact plates.

b. Active Air Monitoring

The method for assessing the microbial quality of air should involve the use of *active* devices such as slit to agar samplers, those using liquid impingement and membrane (or gelatin) filtration, and centrifugal samplers. Each device has certain advantages and disadvantages, although all allow a quantitative testing of the number of organisms per volume of air sampled. The use of such devices in aseptic areas is considered an essential part of evaluating the environment during each production shift, at carefully chosen critical locations. Manufacturers should be aware of a device's air monitoring capabilities, and the air sampler should be evaluated for its suitability for use in an aseptic environment based on cleanability, ability to be sterilized, and disruption of unidirectional airflow. Manufacturers should ensure that such devices are calibrated and used according to appropriate procedures.[10] Because devices vary, the user should assess the suitability of all monitoring devices before they are placed into service.

c. Passive Air Monitoring (Settling Plates)

Another method is the use of passive air samplers, such as settling plates (petri dishes containing nutrient growth medium exposed to the environment). Settling plates lack value as quantitative air monitors because only microorganisms that settle onto the agar surface will be detected. Their value as qualitative indicators in critical areas is enhanced by positioning plates in locations posing the greatest risk of product contamination. As part of methods validation, the quality control laboratory should evaluate what media exposure conditions optimize recovery of low levels of environmental isolates. Exposure conditions should preclude desiccation (e.g., caused by lengthy sampling periods and/or high airflows), which inhibits recovery of microorganisms. The data generated by passive air sampling can be useful when considered in combination with results from other types of air samples.

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[10] For example, the volume of air sampled should be sufficient to yield meaningful measurements of air quality in a given environment.

B. Microbiological Media and Identification

Characterization of recovered microorganisms is an important aspect of the environmental monitoring program. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigation. Monitoring of critical and immediately surrounding clean areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level. In some cases, environmental trending data have revealed migration of microorganisms into the aseptic processing room from either uncontrolled or lesser-controlled areas. Establishing an adequate program for differentiating microorganisms in the lesser-controlled environments, such as Class 100,000 (ISO 8), is instrumental in detecting such trends. At minimum, the program should require species (or, where appropriate, genus) identification of microorganisms in these ancillary environments at frequent intervals to establish a valid, current database of contaminants present in the facility during processing (and to demonstrate that cleaning and sanitization procedures continue to be effective).

Rapid genotypic methods are recommended for purposes of identification, as these methods have been shown to be more accurate and precise than biochemical and phenotypic techniques.

The goal of microbiological monitoring is to reproducibly detect microorganisms for purposes of monitoring the state of environmental control. Consistent methods will yield a database that allows for sound data comparisons and interpretations. The microbiological culture media used in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts and molds) as well as bacteria and incubated at appropriate conditions of time and temperature. Total aerobic bacterial count can be obtained by incubating at 30 to 35°C for 48 to 72 hours. Total combined yeast and mold count is generally obtained by incubating at 20 to 25°C for 5 to 7 days.

Incoming lots of environmental monitoring media should include positive and negative controls. Growth promotion testing should be performed on all lots of prepared media. Where appropriate, inactivating agents should be used to prevent inhibition of growth by cleanroom disinfectants or product residuals (e.g., antibiotics).

C. Prefiltration Bioburden

For any parenteral manufacturing process, prefiltration bioburden should be minimal. In addition to increasing the challenge to the sterilizing filter, high bioburden can contribute endotoxin or other impurities to the drug formulation. An in-process limit for bioburden level for each formulated product (generally sampled immediately preceding sterile filtration) should be established.

D. Alternate Microbiological Test Methods

Other suitable microbiological test methods (e.g., rapid test methods) can be considered for in-process control testing and finished product release testing. We recommend the use of test methods that, upon evaluation, demonstrate increased accuracy, sensitivity, and reproducibility.

E. Particle Monitoring

Routine particle monitoring is useful in rapidly detecting significant deviations in air cleanliness from qualified processing norms (e.g., clean area classification). A result outside the established specifications at a given location should be investigated. The extent of investigation should be consistent with the severity of the *excursion* and include an evaluation of trending data. Appropriate corrective action should be implemented to prevent future deviations.

See Section IV.A for additional guidance on particle monitoring.

XI. STERILITY TESTING

21 CFR 210.3(b)(21) states that "*Representative sample* means a sample that consists of a number of units that are drawn based on rational criteria such as random sampling and intended to assure that the sample accurately portrays the material being sampled."

21 CFR 211.110(a) states, in part, that "To assure batch uniformity and integrity of drug products, written procedures shall be established and followed that describe the in-process controls, and tests, or examinations to be conducted on appropriate samples of in-process materials of each batch. Such control procedures shall be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product."

21 CFR 211.160(b) states that "Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity. Laboratory controls shall include: (1) Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of components, drug product containers, closures, and labeling used in the manufacture, processing, packing, or holding of drug products. The specifications shall include a description of the sampling and testing procedures used. Samples shall be representative and adequately identified. Such procedures shall also require appropriate retesting of any component, drug product container, or closure that is subject to deterioration. (2) Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. Such samples shall be representative and properly identified. (3) Determination of conformance to written descriptions of sampling procedures and appropriate specifications for drug products. Such samples shall be representative and properly identified. (4) The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used."

21 CFR 211.165(a) states that "For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specifications for the drug product, including the identity and strength of each active ingredient, prior to release."

21 CFR 211.165(e) states that "The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with 211.194(a)(2)."

21 CFR 211.167(a) states that "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

21 CFR 211.180(e) states, in part, that "Written records required by this part shall be maintained so that data therein can be used for evaluating, at least annually, the quality standards of each drug product to determine the need for changes in drug product specifications or manufacturing or control procedures."

21 CFR 211.192 states that "All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup."

Certain aspects of sterility testing are of particular importance, including control of the testing environment, understanding the test limitations, and investigating manufacturing systems following a positive test.

The testing laboratory environment should employ facilities and controls comparable to those used for filling and closing operations. Poor or deficient sterility test facilities or controls can result in a high rate of test failures. If production facilities and controls are significantly better than those for sterility testing, the danger exists of mistakenly attributing a positive sterility test result to a faulty laboratory even when the product tested could have, in fact, been nonsterile. Therefore, some manufacturing deficiency may go undetected. We recommend the use of isolators to perform sterility testing. This is a well-established means for minimizing false positives.

A. Choice of Methods

Sterility testing methodologies are required to be accurate and reproducible, in accord with 211.194 and 211.165. The methodology selected should present the lowest potential for yielding a false positive. The USP specifies membrane filtration as the method of choice, when feasible.

As a part of methods validation, appropriate bacteriostasis/fungistasis testing should be conducted. Such testing should demonstrate reproducibility of the method in recovering each of a panel of representative microorganisms. Study documentation should include evaluation of whether microbial recovery from inoculated controls and product samples is comparable throughout the incubation period. If growth is inhibited, modifications (e.g., increased dilution, additional membrane filter washes, addition of inactivating agents) in the methodology should be implemented to optimize recovery. Ultimately, methods validation studies should demonstrate that the methodology does not provide an opportunity for false negatives.

B. Media

It is essential that the media used to perform sterility testing be rendered sterile and demonstrated as growth promoting.

C. Personnel

Personnel performing sterility testing should be qualified and trained for the task. A written program should be in place to regularly update training of personnel and confirm acceptable sterility testing practices.

D. Sampling and Incubation

Sterility tests are limited in their ability to detect low incidences of contamination. For example, statistical evaluations indicate that the USP sterility test sampling plan has been described by USP as "only enabling the detection of contamination in a lot in which 10% of the units are contaminated about nine times out of ten in making the test" (Ref. 13). To further illustrate, if a 10,000-unit lot with a 0.1 percent contamination level was sterility tested using 20 units, there is a 98 percent chance that the batch would pass the test.

This limited sensitivity is why, for batch release purposes, it is important that an appropriate number of units are tested,[11] and that the samples uniformly represent:

- the entire batch – samples should be taken at the beginning, middle, and end of the aseptic processing operation
- the batch processing circumstances – samples should be taken in conjunction with processing interventions or excursions

Because of the limited sensitivity of the test, any positive result is considered a serious CGMP issue that should be thoroughly investigated.

E. Investigation of Sterility Positives

Care should be taken in the performance of the sterility test to preclude any activity that allows for possible sample contamination. When microbial growth is observed, the lot should be considered to be nonsterile and an investigation conducted. It is inappropriate to attribute a positive result to laboratory error on the basis of a retest that exhibits no growth.[12]

Although it is recognized that a determination of whether growth arose from product contamination or laboratory error may not be reached with absolute certainty, it is usually possible to acquire persuasive evidence showing that causative laboratory error is absent. It is difficult to support invalidation of a positive sterility test. Only if conclusive and documented evidence clearly shows that the contamination occurred as part of testing should a new test be performed. When available evidence is inconclusive, batches should be rejected as not conforming to sterility requirements.

After considering all relevant factors concerning the manufacture of the product and testing of the samples, the comprehensive written investigation should include specific conclusions and identify corrective actions. The investigation's persuasive evidence of the origin of the contamination should be based on at least the following:

1. Identification (speciation) of the organism in the sterility test

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[11] USP <71> includes standards for the minimum quantity of units to be analyzed in a valid sterility test.

[12] Underscoring this regulatory standard, USP XXV, section <71>, states that an initial positive test is invalid only in an instance in which "microbial growth can be without a doubt ascribed to" laboratory error (as described in the monograph).

Identification of the sterility test isolate(s) should be to the species level. Microbiological monitoring data should be reviewed to determine if the organism is also found in laboratory and production environments, personnel, or product bioburden. Nucleic acid-based methods are recommended for microbial identification purposes.

2. Record of laboratory tests and deviations

Review of trends in laboratory findings can help to eliminate or implicate the laboratory as the source of contamination. For example, if the organism is seldom found in the laboratory environment, product contamination is likely. If the organism is found in laboratory and production environments, it can still indicate product contamination.

The proper handling of deviations is an essential aspect of laboratory control. When a deviation occurs during sterility testing, it should be documented, investigated, and remedied. If any deviation is considered to have compromised the integrity of the sterility test, the test should be invalidated immediately without incubation.

Deviation and sterility test positive trends should be evaluated periodically (e.g., quarterly, annually) to provide an overview of operations. A sterility positive result can be viewed as indicative of production or laboratory problems and should be investigated globally since such problems often can extend beyond a single batch.

To more accurately monitor potential contamination sources, we recommend you keep separate trends by product, container type, filling line, and personnel. Where the degree of sterility test sample manipulation is similar for a terminally sterilized product and an aseptically processed product, a higher rate of initial sterility failures for the latter should be taken as indicative of aseptic processing production problems.

Microbial monitoring of the laboratory environment and personnel over time can also reveal trends that are informative. Upward trends in the microbial load in the laboratory should be promptly investigated as to cause, and corrected. In some instances, such trends can appear to be more indicative of laboratory error as a possible source of a sterility test failure.

Where a laboratory has a good track record with respect to errors, this history can help remove the lab as a source of contamination since chances are higher that the contamination arose from production. However, the converse is not true. Specifically, where a laboratory has a poor track record, firms should not assume that the contamination is automatically more attributable to the laboratory and consequently overlook a genuine production problem. Accordingly, all sterility positives should be thoroughly investigated.

3. Monitoring of production area environment

Of particular importance is trend analysis of microorganisms in the critical and immediately adjacent areas. Trends are an important tool in the investigation of a sterility failure. Consideration of environmental microbial data should not be limited to results of monitoring the production environment for the lot, day, or shift associated with the suspect lot. For example, results showing little or no recovery of microorganisms can be misleading, especially when preceded or followed by a finding of an adverse trend or atypically high microbial counts. It is therefore important to look at both short- and long-term trend analysis.

4. Monitoring Personnel

Data and associated trends from daily monitoring of personnel should be reviewed and can in some cases strongly indicate a route of contamination. The adequacy of personnel practices and training should also be considered.

5. Product Presterilization Bioburden

Trends in product bioburden should be reviewed (counts and identity). Adverse bioburden trends occurring during the time period of the test failure should be considered during the investigation.

6. Production record review

Complete batch and production control records should be reviewed to detect any signs of failures or anomalies that could have a bearing on product sterility. For example, the investigation should evaluate batch and trending data that indicate whether utility and/or support systems (e.g., HVAC, WFI) are functioning properly. Records of air quality monitoring for filling lines could reveal or show a time at which there was improper air balance or an unusually high particle count.

7. Manufacturing history

The manufacturing history of a product or similar products should be reviewed as part of the investigation. Past deviations, problems, or changes (e.g., process, components, equipment) are among the factors that can provide an indication of the origin of the problem.

XII. BATCH RECORD REVIEW: PROCESS CONTROL DOCUMENTATION

21 CFR 211.100(a) states that “There shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess. Such procedures shall include all requirements in this subpart. These written procedures, including any changes, shall be drafted, reviewed, and approved by the appropriate organizational units and reviewed and approved by the quality control unit.”

21 CFR 211.100(b) states that “Written production and process control procedures shall be followed in the execution of the various production and process control functions and shall be documented at the time of performance. Any deviation from the written procedures shall be recorded and justified.”

21 CFR 211.186 and 211.188 address, respectively, "Master production and control records" and "Batch production and control records."

21 CFR 211.192 states that “All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup.”

Manufacturers should build process and environmental control activities into their aseptic processing operation. It is critical that these activities be maintained and strictly implemented on a daily basis. The requirement for review of all batch records and data for conformance with written procedures, operating parameters, and product specifications prior to arriving at the final release decision for an aseptically processed batch calls for an overall review of process and system performance for that given cycle of manufacture. All in-process data must be included with the batch record documentation in accordance with section 211.188. Review of environmental and personnel monitoring data, as well as other data relating to acceptability of output from support systems (e.g., HEPA / HVAC, WFI, steam generator) and proper functioning of equipment (e.g., batch alarms report; integrity of various filters), should be viewed as essential elements of the batch release decision.

While interventions and/or stoppages are normally recorded in the batch record, the manner of documenting these occurrences varies. In particular, line stoppages and any unplanned interventions should be sufficiently documented in batch records with the associated time and duration of the event. In addition to dwell time of sterile product elements in the critical area, an extensive intervention can increase contamination risk. Sterility failures can be attributed to atypical or extensive interventions that have occurred as a response to an undesirable event during the aseptic process. Written procedures describing the need for line clearances in the event of certain interventions, such as machine adjustments and any repairs, should be established. Such interventions should be documented with more detail than minor events. Interventions that result in substantial activity near exposed product or container closures or that last beyond a reasonable exposure time should, where appropriate, result in a local or full line clearance.

Any disruption in power supply, however momentary, during aseptic processing is a manufacturing deviation and must be included in batch records (211.100, 211.192).

APPENDIX 1: ASEPTIC PROCESSING ISOLATORS

Aseptic processing using isolation systems minimizes the extent of personnel involvement and separates the external cleanroom environment from the aseptic processing line. A well-designed positive pressure isolator, supported by adequate procedures for its maintenance, monitoring, and control, offers tangible advantages over classical aseptic processing, including fewer opportunities for microbial contamination during processing. However, users should not adopt a false sense of security with these systems. Manufacturers should also be aware of the need to establish new procedures addressing issues unique to isolators.

A. Maintenance

1. General

Isolator systems have a number of special maintenance issues. Although no isolator unit forms an absolute seal, very high integrity can be achieved in a well-designed unit. However, a leak in any of certain components of the system can constitute a significant breach of integrity. The integrity of gloves, half-suits, seams, gaskets, and seals should receive daily attention as well as a comprehensive preventative maintenance program. Replacement frequencies should be established in written procedures that ensure parts will be changed before they breakdown or degrade.

2. Glove Integrity

A faulty glove or sleeve (gauntlet) assembly represents a route of contamination and a critical breach of isolator integrity. The choice of durable glove materials, coupled with a well-justified replacement frequency, are two aspects of good manufacturing practice that should be addressed. With every use, gloves should be visually evaluated for any macroscopic physical defect. Mechanical integrity tests should also be performed routinely. An attentive preventive maintenance program can identify and eliminate gloves lacking integrity and will minimize the possibility of placing a sterile product at risk. Such a breach can be of serious consequence.

Due to the potential for microbial migration through microscopic holes in gloves and the lack of a highly sensitive glove integrity test, the inner part of the installed glove should be sanitized regularly and the operator should also wear a second pair of thin gloves.

B. Design

1. Airflow

There are two types of aseptic processing isolators: *open* and *closed*. Closed isolators employ connections with auxiliary equipment for material transfer. Open isolators have openings to the surrounding environment that are carefully engineered to segregate the inner isolator environment from the surrounding room via overpressure.

Turbulent flow is normally acceptable within closed isolators, which are generally compact in size and do not house large processing lines. Other aseptic processing isolators, employ unidirectional airflow that sweeps over and away from exposed sterile materials, avoiding any turbulence or stagnant airflow in the area of exposed sterilized materials, product, and container closures. In most sound designs, air showers over the critical zone once, and then is systematically exhausted. The air handling system should be capable of maintaining the requisite environmental conditions within the isolator.

2. Materials of Construction

As in any aseptic processing design, suitable materials should be chosen based on durability, as well as ease of cleaning and sterilization. For example, rigid wall construction incorporating stainless steel and glass materials is widely used.

3. Pressure Differential

Isolators that include an open exit portal represent a potential compromise in achieving complete physical separation from the external environment. A positive air pressure differential adequate to achieve this full separation should be employed and supported by qualification studies. Positive air pressure differentials from the isolator to the surrounding environment have largely ranged from approximately 0.07" to 0.2" water gauge. The appropriate minimum pressure differential specification established by a firm will depend on the system's design and, when applicable, its exit port. Air balance between the isolator and other direct interfaces (e.g., dry heat tunnel) should also be qualified.

The positive pressure differential should be coupled with appropriate protection at the product egress point(s) to overcome the potential for ingress of any airborne particles from the external environment by induction. Induction can result from local turbulent flow causing air swirls or pressure waves that can push extraneous particles into the isolator. Local Class 100 (ISO 5) protection at an opening can provide a further barrier to induction of surrounding room air into the isolator.

4. Clean Area Classifications

The interior of the isolator should, at minimum, meet Class 100 (ISO 5) standards. The classification of the environment surrounding the isolator should be based on the design of its interfaces (e.g., transfer ports), as well as the number of transfers into and out of the isolator. A Class 100,000 (ISO 8) background can be appropriate depending on isolator design and manufacturing situations. An aseptic processing isolator should not be located in an unclassified room.

C. Transfer of Materials/Supplies

The ability to maintain integrity and sterility of an isolator is impacted by the design of transfer ports. Various adaptations, of differing capabilities, allow for the transfer of supplies into and out of the isolator.

1. General:

Multiple material transfers are generally made during the processing of a batch. Frequently, transfers are performed via direct interface with manufacturing equipment. Properly maintained and operated rapid transfer ports (RTPs) are an effective transfer mechanism for aseptic transfer of materials into and out of isolators. Some transfer ports can have significant limitations, including marginal decontaminating capability (e.g., ultraviolet) or a design that has the potential to compromise isolation by allowing ingress of air from the surrounding room. In the latter case, localized HEPA-filtered unidirectional airflow cover in the area of such a port should be implemented.

2. Discharge

Isolators often include a *mousehole* or other exit port through which product is discharged, opening the isolator to the outside environment. The mousehole represents a potential route of contamination. Sufficient overpressure should be supplied and monitored on a continuous basis at this location to ensure that isolation is maintained.

D. Decontamination

1. Surface Exposure

Written procedures for decontamination of the isolator should be developed. A decontamination process should be developed that provides full exposure of all isolator surfaces to the chemical agent. For example, to facilitate contact with the sterilant, the glove apparatus should be fully extended with glove fingers separated during the decontamination cycle. The interior of the isolator should also be cleaned per appropriate procedures to allow for robust decontamination.

2. Efficacy

A decontamination method should be developed that renders the inner surfaces of the isolator free of viable microorganisms. Decontamination can be accomplished using a number of vaporized agents, although these agents possess limited capability to penetrate obstructed or covered surfaces. Process development and validation studies should include a thorough determination of cycle capability. The characteristics of these agents generally preclude the reliable use of statistical methods (e.g., fraction negative) to determine process lethality (Ref. 14). An appropriate, quantified BI challenge should be placed on various materials[13] and in many locations throughout the isolator, including difficult to reach areas. Cycles should be developed with an appropriate margin of extra kill to provide confidence in robustness of the decontamination processes. Normally, a four- to six-log reduction can be justified depending on the application. The specific BI spore titer used and the selection of BI placement sites should be justified. For example, demonstration of a four-log reduction should be sufficient for introduction of controlled, very low bioburden materials into an aseptic processing isolator, including wrapped sterile supplies that are briefly exposed to the surrounding cleanroom environment.

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[13] If the various isolator materials are thoroughly evaluated during cycle development, a firm might consider placing more focus on material texture and porosity.

The uniform distribution of the defined concentration of decontaminating agent should also be evaluated concurrent with these studies (Ref. 15). Chemical indicators may also be useful as a qualitative tool to show that the decontaminating agent reached a given location.

3. Frequency

While isolators vary widely in design, their interior and content should be designed to be frequently decontaminated. When an isolator is used for multiple days between decontamination cycles, the frequency adopted should include a built-in safety margin and be well justified. This frequency, established during validation studies, should be reevaluated and increased if production data indicate any deterioration of the microbiological quality of the isolator environment.

A breach of isolator integrity should lead to a decontamination cycle. Integrity can be impacted by power failures, valve failure, inadequate overpressure, holes in gloves and seams or other leaks. Breaches of integrity should be investigated and any product that may have been impacted by the breach rejected.

E. Filling Line Sterilization

To ensure sterility of product contact surfaces from the start of each operation, the entire path of the sterile liquid stream should be sterilized. In addition, loose materials or aseptic processing equipment to be used within the isolator should be chosen based on their ability to withstand steam sterilization (or equivalent method). It is expected that materials that permit heat sterilization (e.g., SIP) will be rendered sterile by such methods. Where decontamination methods are used to render certain product contact surfaces free of viable organisms, a minimum of a six-log reduction should be demonstrated using a suitable biological indicator.

F. Environmental Monitoring

An appropriate environmental monitoring program should be established that routinely ensures acceptable microbiological quality of air, surfaces, and gloves (or half-suits) as well as particle levels, within the isolator. Air quality should be monitored periodically during each shift. For example, the exit port should be monitored for particles to detect any unusual results.

G. Personnel

While cleanroom apparel requirements are generally reduced in an isolator operation, the contamination risk contributed by manual factors should not be overlooked. Isolation processes generally include periodic or even frequent use of one or more gloves for aseptic manipulations and handling of material transfers into and out of the isolator. One should be aware that locations on gloves, sleeves, or half suits can be among the more difficult to reach places during surface sterilization, and glove integrity defects may not be promptly detected. Traditional aseptic processing vigilance is appropriate, with an understanding that contaminated isolator gloves can lead to product nonsterility. Accordingly, meticulous aseptic technique standards must be observed (211.113), including appropriate use of sterile tools for manipulations.

APPENDIX 2: BLOW-FILL- SEAL TECHNOLOGY

Blow-fill-seal (BFS) technology is an automated process by which containers are formed, filled, and sealed in a continuous operation. This manufacturing technology includes economies in container closure processing and reduced human intervention, and is often used for filling and packaging ophthalmics and, less frequently, injectables. This appendix discusses some of the critical control points of this technology. Except where otherwise noted below, the aseptic processing standards discussed elsewhere in this document should apply to blow fill seal technology.

A. Equipment Design and Air Quality

Most BFS machines operate using the following steps.

- Heat a plastic polymer resin
- Extrude it to form a parison (a tubular form of the hot resin)
- Cut the parison with a high-temperature knife
- Move the parison under the blow-fill needle (mandrel)
- Inflate it to the shape of the mold walls
- Fill the formed container with the liquid product
- Remove the mandrel
- Seal

Throughout this operation, sterile-air is used, for example, to form the parison and inflate it prior to filling. In most operations, the three steps with the greatest potential for exposure to particle contamination and/or surrounding air are those in which (1) the parison is cut, (2) the parison is moved under the blow-fill mandrel, and (3) the mandrel is removed (just prior to sealing).

BFS machinery and its surrounding barriers should be designed to prevent potential for extraneous contamination. As with any aseptic processing operation, it is critical that contact surfaces be sterile. A validated steam-in-place cycle should be used to sterilize the equipment path through which the product is conveyed. In addition, any other surface with the potential to contaminate the sterile product should be sterile.

The classified environment surrounding BFS machinery should generally meet Class 10,000 (ISO 7) standards, but special design provisions (e.g., isolation technology) can justify an alternate classification. HEPA-filtered or sterile air provided by membrane filters should be used during the steps when sterile products or materials are exposed (e.g., parison formation, container molding or filling steps). Air in the critical area should meet Class 100 (ISO 5) microbiological standards. A well-designed BFS system should also normally achieve Class 100 (ISO 5) airborne particle levels.

Equipment design should incorporate specialized measures to reduce particle levels. In contrast to nonpharmaceutical applications using BFS machinery, control of air quality (i.e., particles) is critical for sterile drug product manufacture. Particles generated during the plastic extrusion, cutting, and sealing processes should be controlled. Provisions for carefully controlled airflow can protect the product by forcing generated particles outward while preventing any ingress from the adjacent environment. Furthermore, designs separating the filling zone from the surrounding environment are important to ensure product protection. Barriers, pressure vacuums, microenvironments, and appropriately directed high velocities of sterile air have been found useful in preventing contamination (Ref. 16). Smoke studies and multi-location particle data can provide valuable information when performing qualification studies to assess whether proper particle control dynamics have been achieved throughout the critical area.

In addition to suitable design, an adequate preventative maintenance program should be established. For example, because of its potential to contaminate the sterile drug product, the integrity of the cooling or boiling system (e.g., mold plates, gaskets) should be carefully monitored and maintained.

B. Validation/Qualification

Advantages of BFS processing are known to include rapid container closure processing and minimized aseptic interventions. However, only a properly functioning process can realize these advantages. Setup, troubleshooting of equipment, and related aseptic personnel procedures should be given special attention. Equipment sterilization, media fills, polymer sterilization, endotoxin removal, product-plastic compatibility, forming and sealing integrity, and unit weight variation are among the key issues that should be covered by validation and qualification studies.

Appropriate data should ensure that BFS containers are sterile and, if used for parenteral drugs, nonpyrogenic. This can generally be achieved by validating that time temperature conditions of the extrusion process are effective against endotoxin or spore challenges in the polymeric material.

The plastic polymer material chosen should be pharmaceutical grade, safe, pure, and pass appropriate criteria (Ref. 17) for plastics. Polymer suppliers should be qualified and monitored for raw material quality.

C. Batch Monitoring and Control

In-process monitoring should include various control parameters (e.g., container weight variation, fill weight, leakers, air pressure) to ensure ongoing process control. Microbial air quality is particularly important. Samples should be taken per a comprehensive sampling plan that provides data representative of the entire filing operation. Continuous monitoring of particles can provide valuable data relative to the control of a blow-fill-seal operation.

Container closure defects can be a major problem in control of a BFS operation. It is critical that the operation be designed and set-up to uniformly manufacture leak-proof units. As a final measure, the inspection of each unit of a batch should include a reliable, sensitive, final product examination that is capable of identifying defective units (e.g., *leakers*). Significant defects due to heat or mechanical problems, such as mold thickness, container or closure interface deficiencies, poorly formed closures, or other deviations should be investigated in accord with §§ 211.100 and 211.192.

APPENDIX 3: PROCESSING PRIOR TO FILLING AND SEALING OPERATIONS

The purpose of this appendix is to supplement the guidance provided in this document with information on products regulated by CBER or CDER that are subject to aseptic processing at points early in the manufacturing process, or that require aseptic processing through the entire manufacturing process because it is impossible to filter sterilyze the final drug product. The scope of this appendix includes aseptic processing activities that take place prior to the filling and sealing of the finished drug product. Special considerations include those for:

A. Aseptic processing from early manufacturing steps

Some products should undergo aseptic processing at some or all manufacturing steps preceding the final product closing step. With some products, there is a point in the process after which a product can no longer be rendered sterile by filtration. In such cases, the product would be handled aseptically at all steps subsequent to filter sterilization. In other instances, the final drug product cannot be filter sterilyzed, and, therefore, each component in the formulation would be rendered sterile and mixed aseptically. For example, products containing aluminum adjuvant are formulated aseptically because once they are alum adsorbed, they cannot be sterile-filtered.

When a product is processed aseptically from the early stages, the product and all components or other additions are rendered sterile prior to entering the manufacturing process. It is critical that all transfers, transports, and storage stages be carefully controlled at each step of the process to maintain sterility of the product.

Procedures (e.g., aseptic connection) that expose a product or product contact surfaces should be performed under unidirectional airflow in a Class 100 (ISO 5) environment. The environment of the room surrounding the Class 100 (ISO 5) environment should be Class 10,000 (ISO 7) or better. Microbiological and airborne particle monitoring should be performed during operations. Microbial surface monitoring should be performed at the end of operations, but prior to cleaning. Personnel monitoring should be performed in association with operations.

Process simulation studies should be designed to incorporate all conditions, product manipulations, and interventions that could impact on the sterility of the product during manufacturing. The process simulation, from the early process steps, should demonstrate that process controls are adequate to protect the product during manufacturing. These studies should incorporate all product manipulations, additions, and procedures involving exposure of product contact surfaces to the environment. The studies should include worst-case conditions such as maximum duration of open operations and maximum number of participating operators. However, process simulations do not need to mimic total manufacturing time if the manipulations that occur during manufacturing are adequately represented.

It is also important that process simulations incorporate storage of product or transport to other manufacturing areas. For instance, there should be assurance of bulk vessel integrity for specified holding times. The transport of bulk tanks or other containers should be simulated as part of the media fill. Please refer to Section IX.A for more guidance on media simulation studies. Process simulation studies for the formulation stage should be performed at least twice per year.

B. Aseptic processing of cell-based therapy products (or of products intended for use as cell based therapies)

Cell-based therapy products represent a subset of the products for which aseptic manipulations are used throughout the process. Where possible, closed systems should be used during manufacturing. Cell-based therapy products often have short processing times at each manufacturing stage, even for the final product. Often, these products are administered to patients before final product sterility testing results are available. In situations where results of final sterility testing are not available before the product is administered, additional controls and testing should be considered. For example, additional sterility tests can be performed at intermediate stages of manufacture, especially after the last manipulation of the product prior to administration. Other tests that may indicate microbial contamination, such as microscopic examination, gram stains, and endotoxin testing should be performed prior to product release.

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Contains Nonbinding Recommendations

RELEVANT GUIDANCE DOCUMENTS

Some relevant FDA guidance documents include:

- Guidance for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products
- Guideline for Validation of Limulus Amebocyte Lysate Test as an End Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices
- Guide to Inspections of Lyophilization of Parenterals
- Guide to Inspections of High Purity Water Systems
- Guide To Inspections of Microbiological Pharmaceutical Quality Control Laboratories
- Guide To Inspections of Sterile Drug Substance Manufacturers
- Pyrogens: Still a Danger; (Inspection Technical Guide)
- Bacterial Endotoxins/Pyrogens; (Inspection Technical Guide)
- Heat Exchangers to Avoid Contamination; (Inspection Technical Guide)
- See also the draft guidance *Container and Closure Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products*, which was issued in 1998. Once final, it will represent the Agency's thinking on this topic.

GLOSSARY

Air lock- A small room with interlocked doors, constructed to maintain air pressure control between adjoining rooms (generally with different air cleanliness standards). The intent of an aseptic processing airlock is to preclude ingress of particulate matter and microorganism contamination from a lesser controlled area.

Alert Level- An established microbial or airborne particle level giving early warning of potential drift from normal operating conditions and triggers appropriate scrutiny and follow-up to address the potential problem. Alert levels are always lower than action levels.

Action Level- An established microbial or airborne particle level that, when exceeded, should trigger appropriate investigation and corrective action based on the investigation.

Aseptic Processing Facility- A building containing cleanrooms in which air supply, materials, and equipment are regulated to control microbial and particle contamination.

Aseptic Processing Room- A room in which one or more aseptic activities or processes is performed.

Asepsis- A state of control attained by using an aseptic work area and performing activities in a manner that precludes microbiological contamination of the exposed sterile product.

Bioburden- The total number of microorganisms associated with a specific item prior to sterilization.

Barrier- A physical partition that affords aseptic manufacturing zone protection by partially separating it from the surrounding area.

Biological Indicator (BI)- A population of microorganisms inoculated onto a suitable medium (e.g., solution, container or closure) and placed within appropriate sterilizer load locations to determine the sterilization cycle efficacy of a physical or chemical process. The *challenge microorganism* is selected based upon its resistance to the given process. Incoming lot D-value and microbiological count define the quality of the BI.

Clean Area- An area with defined particle and microbiological cleanliness standards.

Cleanroom- A room designed, maintained, and controlled to prevent particle and microbiological contamination of drug products. Such a room is assigned and reproducibly meets an appropriate air cleanliness classification.

Component- Any ingredient intended for use in the manufacture of a drug product, including those that may not appear in the final drug product.

Colony Forming Unit (CFU)- A microbiological term that describes the formation of a single macroscopic colony after the introduction of one or more microorganisms to microbiological growth media. One colony forming unit is expressed as 1 CFU.

Critical Area - An area designed to maintain sterility of sterile materials. Sterilized product, containers or closures, and equipment may be exposed in critical areas.

Clean Zone- See Clean Area.

Critical surfaces- Surfaces that may come into contact with or directly affect a sterilized product or its containers or closures. Critical surfaces are rendered sterile prior to the start of the manufacturing operation, and sterility is maintained throughout processing.

Decontamination- A process that eliminates viable bioburden via use of sporicidal chemical agents.

Depyrogenation- A process used to destroy or remove pyrogens (e.g., endotoxin).

D value- The time (in minutes) of exposure at a given temperature that causes a one-log or 90 percent reduction in the population of a specific microorganism.

Dynamic- Conditions relating to clean area classification under conditions of normal production.

Endotoxin- A pyrogenic product (e.g., lipopolysaccharide) present in the bacterial cell wall. Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.

Gowning Qualification- A program that establishes, both initially and on a periodic basis, the capability of an individual to don the complete sterile gown in an aseptic manner.

HEPA filter- High efficiency particulate air filter with minimum 0.3 micron particle retaining efficiency of 99.97 percent.

HVAC- Heating, ventilation, and air conditioning.

Intervention- An aseptic manipulation or activity that occurs at the critical zone.

Isolator- A decontaminated unit, supplied with Class 100 (ISO 5) or higher air quality, that provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding clean room air and personnel). There are two major types of isolators:

> *Closed isolator systems* exclude external contamination from the isolator's critical zone by accomplishing material transfer via aseptic connection to auxiliary equipment, rather than use of openings to the surrounding environment. Closed systems remain sealed throughout operations.

> *Open isolator systems* are designed to allow for the continuous or semi-continuous ingress and/or egress of materials during operations through one or more openings. Openings are engineered (e.g., using continuous overpressure) to exclude the entry of external contamination into the isolator.

Laminar flow- An airflow moving in a single direction and in parallel layers at constant velocity from the beginning to the end of a straight line vector.

Operator- Any individual participating in the aseptic processing operation, including line set-up, filler, maintenance, or other personnel associated with aseptic line activities.

Overkill sterilization process- A process that is sufficient to provide at least a 12 log reduction of microorganisms having a minimum D value of 1 minute.

Pyrogen- A substance that induces a febrile reaction in a patient.

Sterile Product- For purposes of this guidance, *sterile product* refers to one or more of the elements exposed to aseptic conditions and ultimately making up the sterile finished drug product. These elements include the containers, closures, and components of the finished drug product.

Sterilizing grade filter- A filter that, when appropriately validated, will remove all microorganisms from a fluid stream, producing a sterile effluent.

Unidirectional flow- An airflow moving in a single direction, in a robust and uniform manner, and at sufficient speed to reproducibly sweep particles away from the critical processing or testing area.

Terminal sterilization- The application of a lethal agent to sealed, finished drug products for the purpose of achieving a predetermined sterility assurance level (SAL) of usually less than 10^{-6} (i.e., a probability of a nonsterile unit of greater than one in a million).

ULPA filter- Ultra-low penetration air filter with minimum 0.3 micron particle retaining efficiency of 99.999 percent.

Validation- Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

Worst case- A set of conditions encompassing upper and lower processing limits and circumstances, including those within standard operating procedures, that pose the greatest chance of process or product failure (when compared to ideal conditions). Such conditions do not necessarily induce product or process failure.